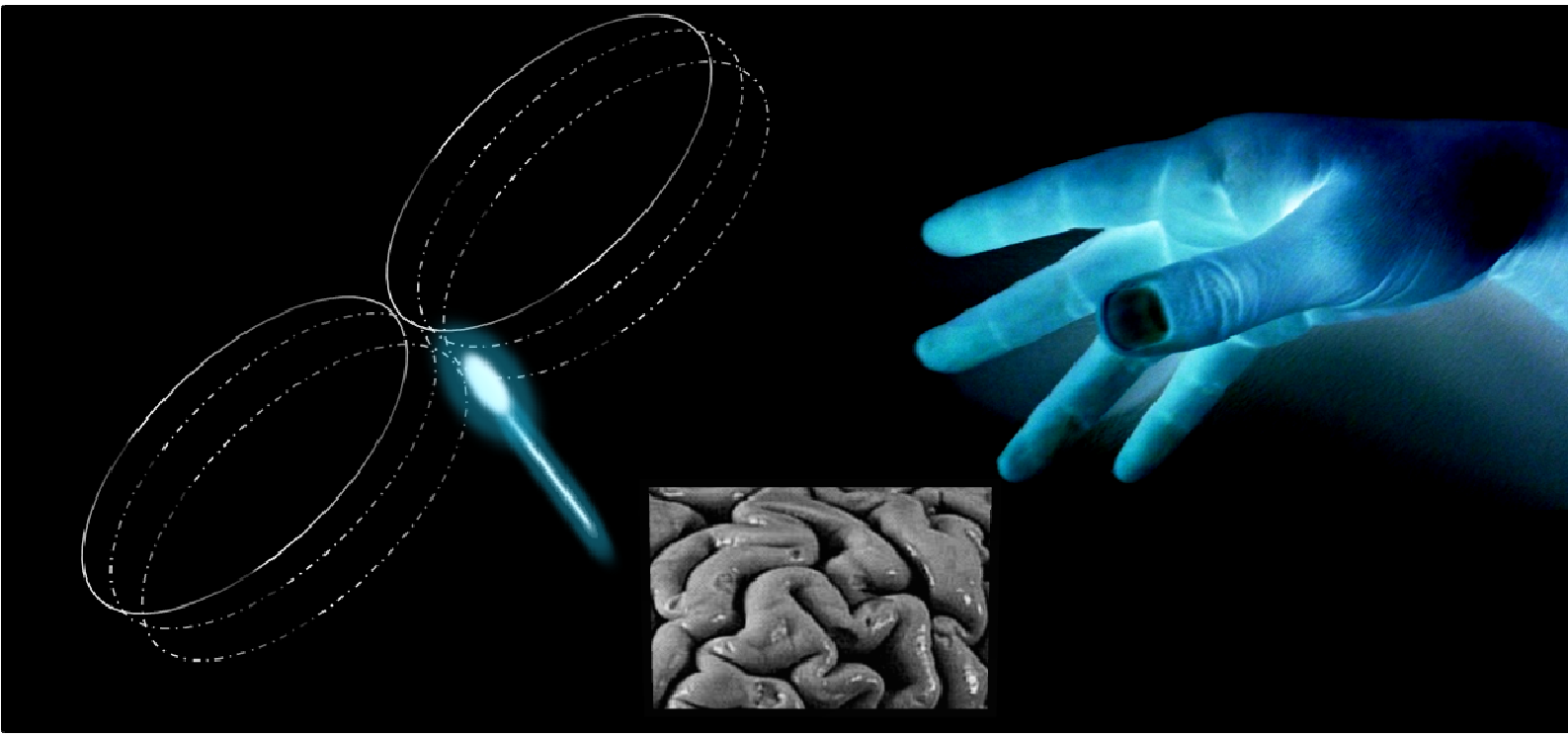


Plasticity of the interregional connectivity of the primary motor cortex

A neurophysiological study by non-invasive brain stimulation

DOCTORAL THESIS

Guadalupe Nathzidy Rivera Urbina

Plasticity of the interregional connectivity of the primary motor cortex

A neurophysiological study by non-invasive brain stimulation

Guadalupe Nathzidy Rivera-Urbina

Doctoral Thesis directed by

Agnès Gruart i Massó and Andrés Molero Chamizo

Sevilla, 2014

Doña Agnès Gruart i Massó, Catedrática de Fisiología en el Departamento de Fisiología, Anatomía y Biología Celular de la Universidad Pablo de Olavide (Sevilla) y Don Andrés Molero Chamizo, Profesor Titular de Universidad del área de Psicobiología, adscrita al Departamento de Psicología Clínica, Experimental y Social de la Universidad de Huelva:

CERTIFICAN

que el presente trabajo titulado "**Plasticity of the interregional connectivity of the primary motor cortex. A neurophysiological study by non-invasive brain stimulation"** ha sido realizado bajo su dirección y supervisión por Doña Guadalupe Nathzidy Rivera-Urbina, graduada en Psicología por la Universidad del Valle de México. El título ya ha sido homologado en España. Los directores consideran que la tesis reúne las condiciones y el rigor científico para ser presentado y defendido como Tesis Doctoral.

Sevilla, 2 de Septiembre de 2014

Fdo.: Agnès Gruart i Massó Fdo.: Andrés Molero Chamizo

DEDICATORIAS

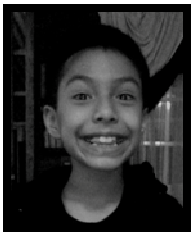
Para y por ustedes…

¡Santiago! Pero, y ahora, ¿Qué es eso? ¿Qué vas a hacer con eso? Preguntaba ella, mi abuela, con la misma expresión de asombro con la que mis ojos veían a mi abuelo entrar por la puerta grande de la casa de la esquina, empujando un gran pedazo de chatarra. -Es un coche- contestó él. Eso no podía ser un coche, quizá pudo haber sido un coche pero hacía mucho pero que mucho tiempo, pensé. Doña Lu, se lo dijo, eso no es un coche. Él, contestó inmediatamente - lo es, yo lo voy a armar-. Fue el último de tus proyectos y tal vez el mejor de mis regalos fue verte trabajar cada tarde y mirar con asombro cómo poco a poco el pedazo de chatarra se transformaba frente a mis ojos en un coche.

Por tu trabajo diario, por tu paciencia eterna disfrazada de regaños, por la tardes, por los días y las noches a tu lado, por la confianza, por defenderme siempre, por creer en mí, por tu lucha, por tu entrega, por el tiempo que no vuelve, por tu amor sin condiciones.

Señora Paty le llaman algunos, y sólo Paty los que le hablan con más confianza, Yo, le llamo mamá y, gracias a esa circunstancia de la vida, observaba día a día a una de las personas más audaces, constantes e inteligentes que he conocido, para desfortuna mía, yo no soy buena alumna. Para mis adentros siempre reconozco que ella siempre, cada cosa la habría hecho mejor.

Vimos juntos por primera vez el mar, además de ese recuerdo, hemos compartido algunas cosas más: películas, juegos, juguetes, paseos, peleas, reconciliaciones, regaños, comidas, regalos, programas de televisión, clases de patinaje, de karate, de gimnasia, de natación, ideas, platicas, varios perros, incluso podría asegurar que el perro favorito de los tres es el mismo. Sí, hemos compartido algunas cosas, la misma casa, la misma mesa, la vida misma. El tiempo siempre fue, es y será mejor a su lado…



Por tu sonrisa, por tu energía, porque seas feliz siempre…

Esta tesis como algunas otras está cargada de cafés en solitario, pero también compartidos, días de laboratorio, otros tantos de lectura, de ordenador, muchos momentos de desesperanza, desesperación, algunas ideas fugaces de abandono, también tiene mucho de nostalgia de la que gente como ustedes que de alguna u otra forma, estuvieron ahí para apoyarme. **Gracias…**

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A mis amigas **Gaby Méndez y Justina Sosa** por estar siempre.

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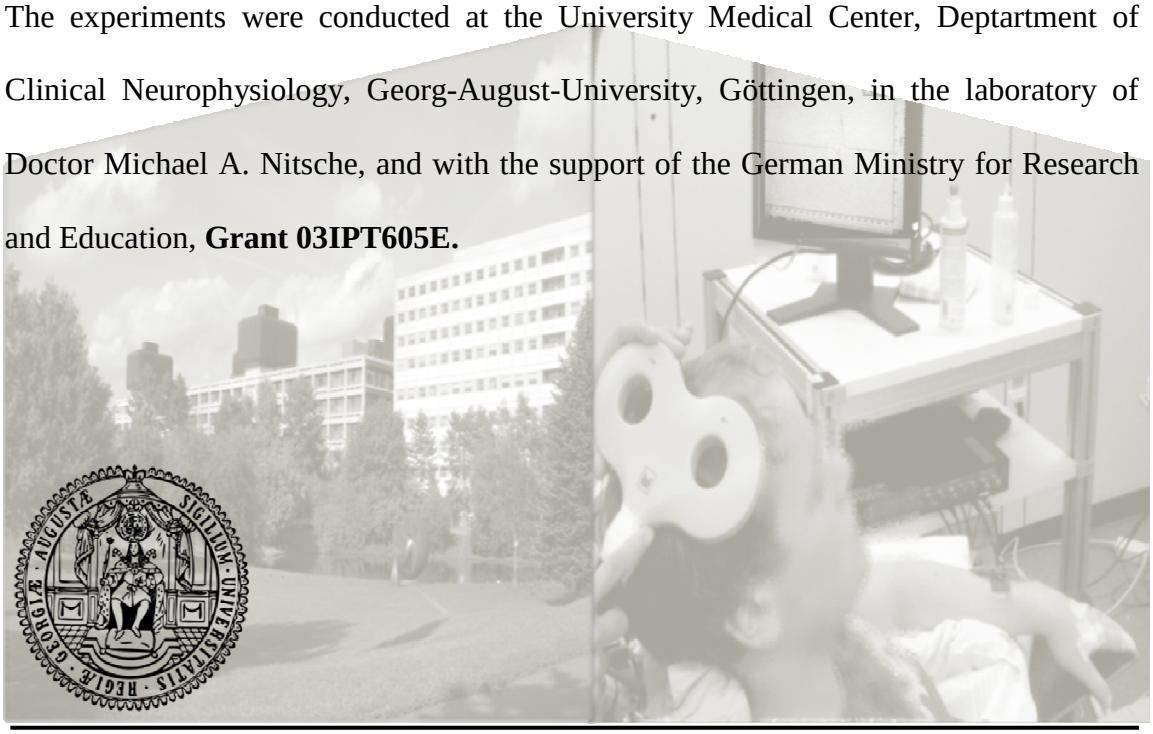

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ABBREVIATIONS

%MSO	percentage of maximum stimulator output
AMT	active motor threshold
CS	conditioning stimulus
DTI	diffusion tensor imaging
EEG	electroencephalography
FDA	Food and Drugs Administration
FDI	first dorsal interosseous
ICF	intracortical facilitation
ICI	intracortical inhibition
I-O curve	input-output curve
ISI	interstimulus interval
M1	primary motor cortex
MEP	motor evoked potential
MT	motor threshold
NIBS	non invasive brain stimulation
P3	posterior parietal cortex region of the international 10-20 electroencephalography system
PPC	posterior parietal cortex
PM	premotor
pmICF	parieto-motor intracortical facilitation
pmSICI	parieto-motor short latency intracortical inhibition
REM	rapid eye movements
RMT	resting motor threshold
SICI	short-latency intracortical inhibition
SLF	superior longitudinal fasciculus
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
TS	test stimulus

ABSTRACT

Background: The posterior parietal cortex is part of the cortical network involved in motor learning and is structurally and functionally connected with the primary motor cortex. Neuroplastic alterations of neuronal connectivity might be an important basis for learning processes. These have however not been explored for parieto-motor cortical connections in humans.

Objective: We aimed to explore plastic alterations of parieto-motor cortical connections by transcranial direct current stimulation (tDCS) in healthy humans through four different experiments.

Methods: In the first experiment, fourteen subjects received anodal and cathodal tDCS over the left posterior parietal cortex for 15 min (electrode position P3, stimulation intensity 0.5 mA, electrode size 15 cm^2), six of them also received sham tDCS. In the second experiment, thirteen subjects received anodal, cathodal and sham tDCS over P3, and 3 cm posterior or lateral to P3 to explore spatial specificity of the effects. Subsequent neuroplastic changes of cortico-spinal excitability were monitored via motor evoked potentials (MEP) and Input-Output curve (I-O curve) elicited by single pulse transcranial magnetic stimulation (TMS). In the third experiment, fifteen subjects received anodal, cathodal and sham tDCS over P3, and short latency intracortical inhibition/intracortical facilitation (SICI/ICF) over the primary motor cortex were recorded before and after P3 tDCS. In the last experiment, parieto-motor short latency intracortical inhibition/intracortical facilitation (pmSICI/pmICF) were obtained via parieto-motor cortex TMS conducted by a paired-pulse twin coil TMS protocol before and after P3 tDCS.

Results: The results show tDCS-induced polarity-dependent motor cortex excitability alterations primarily after tDCS over P3. Single pulse-TMS-elicited MEP, motor cortex SICI/ICF at 5-7 ms and 10-15 ms interstimulus intervals (ISIs), parieto-motor cortex intracortical excitability at 10-15 ms ISIs were enhanced by P3 anodal stimulation. Single pulse-TMS-elicited MEP, and parieto-motor cortex intracortical excitability at ISI 10-15 ms were reduced by parietal cathodal tDCS. Cortico-spinal excitability alterations lasted for at least 120 min after stimulation.

*Conclusio*n: These results show an effect of remote stimulation of parietal areas on primary motor cortex excitability. The spatial specificity of the effects as well as the impact on parieto-motor cortex connections are in accordance with an at least partially connectivity-driven effect.

Keywords: Motor cortex; Parietal cortex; Transcranial direct current stimulation; Transcranial magnetic stimulation

RESUMEN

Antecedentes: La corteza parietal posterior es parte de la red cortical relacionada con el aprendizaje motor y está estructuralmente y funcionalmente conectada con la corteza motora primaria. Los cambios neuroplásticos de la conectividad neuronal entre estas dos estructuras podrían ser una base importante para el proceso de aprendizaje. Sin embargo, estos cambios entre las conexiones parieto-motoras no han sido explorados en humanos.

Objetivos: El objetivo de esta investigación se centra en estudiar los cambios de las conexiones parieto-motoras, utilizando estimulación con corriente directa transcraneal (tDCS) en humanos sanos, mediante cuatro diferentes experimentos.

Métodos: En el primer experimento se utilizó una muestra de 14 participantes, quienes recibieron tDCS anodal y catodal sobre la corteza parietal posterior (P3) por un periodo de 15 minutos, a una intensidad de 0.5 mA, utilizando un electrodo de 15 cm^2. Además, seis de ellos formaron el grupo control, quienes recibieron falsa estimulación. En el segundo experimento, 13 participantes recibieron tDCS anodal, catodal y falsa estimulación sobre P3, y 3 cm posterior o lateral a P3 con el fin de explorar la especificidad espacial de los efectos. Los cambios neuroplásticos de la excitabilidad cortico-espinal subsecuentes se monitorizaron a través del registro de los potenciales evocados motores (MEP) inducidos mediante estimulación magnética transcraneal (TMS) de pulsos simples. En el tercer experimento, 15 participantes recibieron tDCS anodal, catodal y falsa estimulación sobre P3. Se registró la inhibición intracortical de intervalo corto y la facilitación intracortical (SICI/ICF) de la corteza motora primaria mediante TMS, antes y después de P3 tDCS. En el último experimento se registró la inhibición intracortical de intervalo corto/facilitación intracortical parieto-motora

(pmSICI/pmICF), mediante parieto-motor TMS utilizando un protocolo de TMS de doble bobina con pulsos pareados, antes y después de P3 tDCS.

Resultados: Los resultados muestran alteraciones de la excitabilidad de la corteza motora dependientes de la polaridad inducidas mediante tDCS, primariamente después de tDCS sobre P3. Los PEM inducidos mediante TMS de pulsos simples, la SICI/ICF de la corteza motora a los 5-7 ms y 10-15 ms intervalos inter estímulos (ISIs), y la excitabilidad intracortical parieto motora a los 10-15 ms ISIs aumentaron tras anodal tDCS sobre P3. Los PEM inducidos mediante TMS de pulsos simples, y la excitabilidad intracortical parieto-motora a los 10-15 ms ISIs se redujeron mediante parietal catodal tDCS. Las modificaciones de la excitabilidad cortico-espinal tuvieron una duración de hasta 120 minutos después de la estimulación.

Conclusiones: Estos resultados muestran un efecto de estimulación a distancia de las zonas parietales sobre la excitabilidad de la corteza motora primaria. La especificidad espacial de los efectos, así como el impacto en las conexiones de la corteza parietal-motora son compatibles, al menos parcialmente, con un efecto derivado de la conectividad cortical.

1. INTRODUCTION

Primary motor cortex has anatomical and functional connectivity with other cortical areas; this efferent network infallibly plan, prepare and order every voluntary movement. Each movement is preceded by testing intrinsic and extrinsic information for accurate movement within space. The involvement of the parietal cortex in this process is essential. In 2007, Koch et al. showed that stimulation of the posterior parietal cortex increased the excitability of the ipsilateral motor cortex. Thus, they demonstrated the existence of facilitatory parieto-motor connectivity. Later, Karabanov et al. (2013) corroborated that parietal cortex has facilitatory and inhibitory connections to the primary motor cortex. Parieto-motor network is critical to execute accurate movements. Nevertheless, the neurophysiologic mechanisms of neuronal connectivity in voluntary movements need to be elucidated.

Transcranial magnetic stimulation is a non invasive tool to explore cortical motor function in healthy subjects and in neurological disorders as well. In recent studies, transcranial magnetic stimulation has been used to probe connectivity between cortical areas (Rothwell, 2011). To evaluate motor cortex excitability with this technique, there are several protocols (Ziemann et al., 2008). On the other hand, trasncranial direct current stimulation is a non-invasive neuromodulation tool to induce changes in cortical excitability (Knotkova et al., 2013). The combination of both non invasive brain stimulation techniques allows exploring the plasticity in cortical areas (Reis et al., 2008). This thesis aims to explore the connectivity between posterior parietal cortex (P3) and primary motor cortex, using non-invasive brain stimulation techniques.

Transcranial magnetic stimulation protocols recorded in this thesis in healthy and right handed subjects were: motor-evoked potentials, resting motor threshold, active

motor threshold, input-output curve, short-latency intracortical inhibition, intracortical facilitation and parieto-motor short-latency intracortical inhibition, intracortical facilitation paired-pulse twin coil. These protocols were used to record the baseline motor cortex excitability. After that, transcranial direct current stimulation was applied over posterior parietal cortex region (P3) of the international 10-20 electroencephalography system, or 3 cm posterior or lateral to P3 (15 min, 0.5 mA), and different protocols were recorded in different experiments to monitor motor cortex evoked potentials before and after stimulation.

The results of this thesis show facilitatory and inhibitory motor cortex changes induced by anodal and cathodal transcranial magnetic stimulation over posterior parietal cortex. These findings help to elucidate the neurophysiological mechanisms related to the functional and anatomical connectivity between primary motor and posterior parietal cortices, and might help to clarify the specific changes of the cortical connectivity necessary to motor learning, for example in relation to the preparation for a forthcoming movement.

1.1 PRIMARY MOTOR CORTEX

Motor cortex is located in the frontal lobe, immediately anterior to the central sulcus and over the temporal lobe. Motor cortex compromises 4 areas: primary motor cortex (M1 or Brodmann's area 4), premotor cortex (Brodmann's area 6 lateral), supplementary motor area (Brodmann's area 6 medial) and frontal eye fields (Brodmann's area 8).

Motor cortex is an efferent structure responsible of the voluntary movements on the contra-lateral side of the body. Neurons in the motor cortex plan, start and coordinate sequences of voluntary movements. Neurons in primary motor cortex have an association with movement direction, and the force, extent and speed of movements (Figure 1A). However, nowadays the evidence points to the fact that the voluntary movements are the result of distributed motor networks.

1.1.1 Anatomical description

Primary motor cortex is part of the motor area; is located anterior to the central sulcus and posterior to the supplementary motor area (medially) and premotor cortex (laterally) (Figure 1B). Traditionally, the primary motor cortex has been considered as containing a somatotopical representation of the musculature. This representation has been schematized by the homunculus (Figure 1C), so that the muscles associated with fine and discriminative movements as hands, lips, tongue, etc., have a lager somatotopic representation (Penfield and Bolchey, 1937).

Some studies expose an overlap in the functional organization of primary motor cortex. Multiple sites of activation can be observed by functional magnetic resonance imaging in the primary motor cortex for finger movements (Sanes et al., 1995; Sanes and Schieber, 2001).

The commands to perform any voluntary movement are guided by primary motor cortex, via axons of pyramidal neurons that descend to local circuits in the brainstem (pyramidal corticobulbar tract) and spinal cord (pyramidal corticospinal tract). Both tracts are descending pathways.

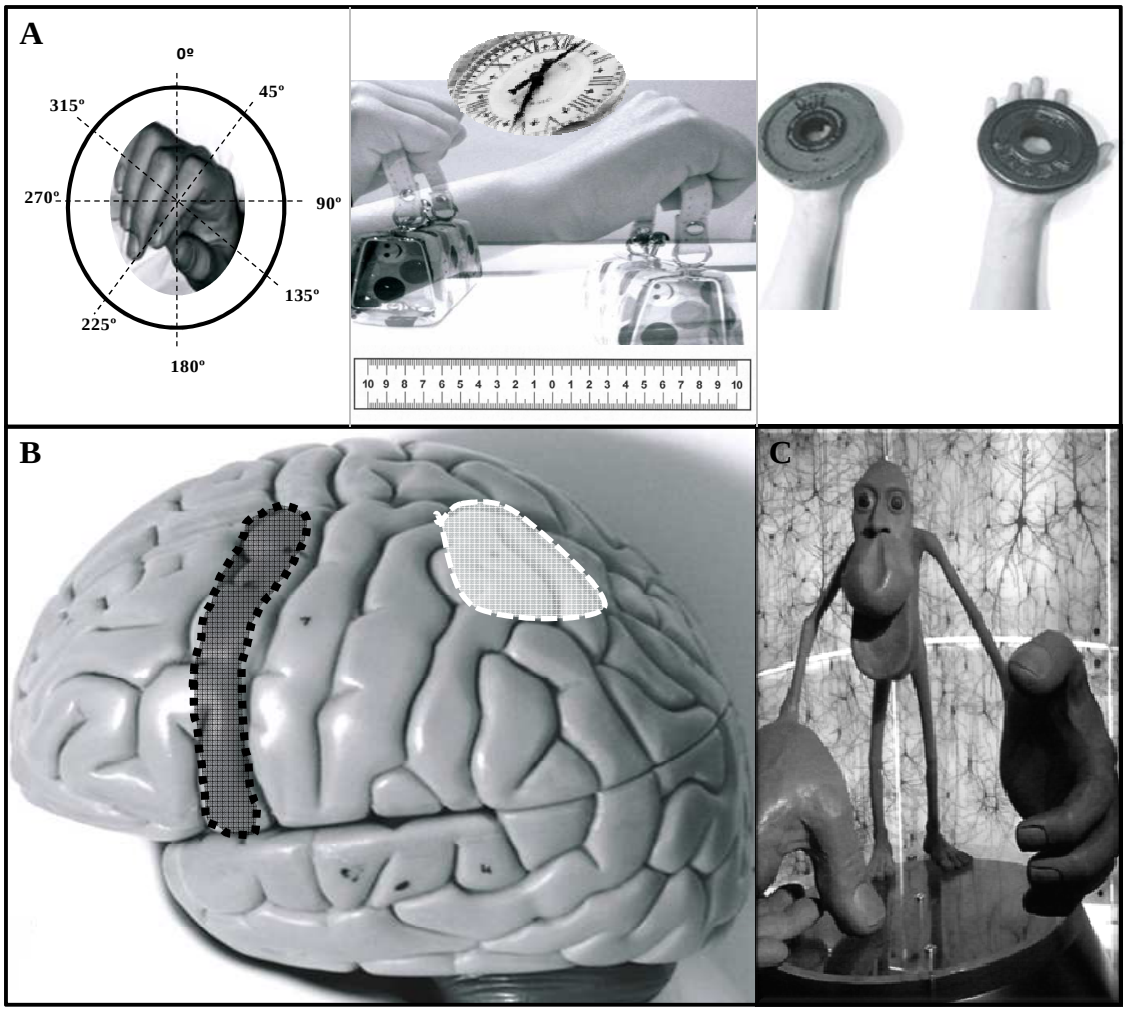


Figure 1. Primary motor cortex and posterior parietal cortex. Primary motor cortex is functionally associated with the movement direction, and the extent, speed and force of the movements (**A**). Primary motor cortex is located anterior to the central sulcus (**B**: dotted black line). Posterior parietal cortex is located posterior to the primary somatosensory cortex (**B**: dotted white line). Human muscles are associated to different areas of the primary motor cortex. A graphical somatotopic representation of the body muscles on the motor cortex is known as motor homunculus (**C**).

Cerebral cortices are organized into cell layers. Laminar organization can be different in the various cortical regions; the most typical organization of the layers in the cortex contains six layers. Primary motor cortex has essentially no internal granule cell layer (layer IV), and thus is called agranular cortex. The leanness of layer IV can be explained in relation to its connections with the thalamus. Layer IV is the main target of

sensory information arriving from the thalamus, but the motor cortex is an output region that otherwise has a more prominent output layer V.

The layered organization provides to the cerebral cortex the capacity to receive inputs and to send outputs. Cerebral cortex receives inputs from subcortical structures and other cortical regions on both hemispheres. Cerebral cortex directs outputs to several subcortical and cortical regions, including other regions of the brain on both hemispheres. The layers in the cerebral cortex have a very efficient and organized input-output relationship.

1.1.2 Primary motor cortex and posterior parietal cortex: Cortical connections

Posterior parietal cortex is located in the parietal lobe, posterior to the primary somatosensory parietal cortex (Brodmann's area 3, 1, 2). Posterior parietal cortex is formed by the Brodmann's areas number 5 and 7 (Figure 1B). Neurons in these areas are involved in different types of higher-order somatosensory processing: they are direction-sensitive, orientation-sensitive, texture-sensitive, and shape-sensitive neurons.

Anatomical connectivity in the cerebral cortex is mediated by white matter; formed by axons which tend to form fasciculus. Figure 2 shows the superior longitudinal fasciculus which connect the posterior parietal cortex with frontal areas, including primary motor cortex (Dejerine, 1895). As mentioned above, sensory cortices tend to have a dense layer of granule cells (layer IV) compared to motor cortex. When a hand is manipulating an object, posterior parietal cortex lets the integration with other senses and the sensitive integration of all the body is formed.

Functionally, sensory and motor systems interact all the time. Information between egocentric reference frames to facilitate sensory-guided action, or from egocentric to allocentric reference frames to facilitate spatial navigation, are necessary to execute accurate movements. However, how these interchanges of information occur between reference frames and what areas mediate each transformation is poorly understood in the human brain (Szczepanski and Saalmann, 2013).

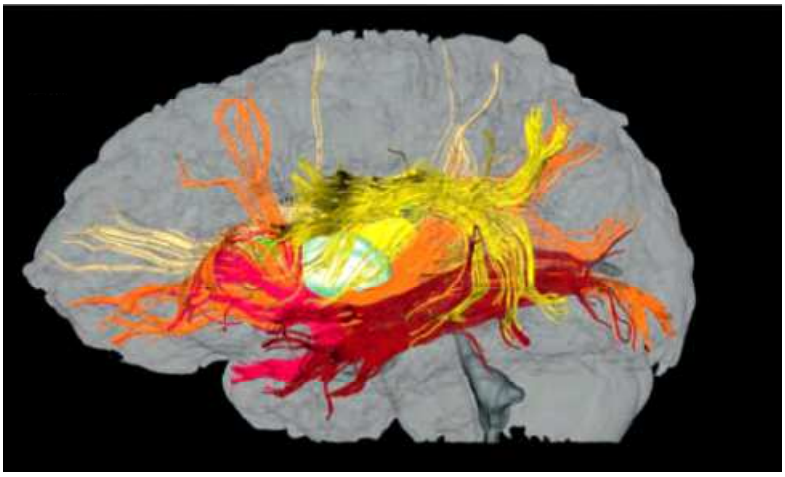


Figure 2. Axonal tracts delineated by diffusion tensor imaging (DTI). The yellow tract represents the superior longitudinal fasciculus which connects the posterior parietal area with frontal areas. Image reproduced with permission from Mori et al., 2009.

Gharbawie et al. (2012) performed a study in macaques using electrical intracortical stimulation. They could evoke movements from parietal cortex by that stimulation, and found that the primary motor cortex cells related to grasp were extended to the anterior parietal cortex (71%) and posterior parietal cortex (22%). Most of those zones were directly targeted to the grasp zone. Another recent study realized with seven adult marmoset monkeys found that a considerable proportion of the total

ipsilateral input of the primary motor cortex was originated in the posterior parietal cortex. The densest projections were targeted to the medial and intermediate parts of primary motor cortex, which are related to the limbs control (Burman et al., 2014). A projection from posterior parietal cortex to primary motor cortex seems to be related to the control of limbs movements, with respect to interaction with stimulus in peripersonal space. Luria (1966) found that soldiers who had been injured in the war with bilateral injuries to the posterolateral parietal lobe had normal visual acuity but were unable to scan visually or reach for an object of interest. When asked to describe what they saw, the wounded soldiers could not put together the elements of a visual scene. These studies showed that the posterior association areas are critical for integrating different sensory modalities. Definitely frontal parietal network is essential in manipulating environment information to define a precise relation between the space around us and make accurate movements.

1.2 CORTICAL PLASTICITY AND NON-INVASIVE BRAIN STIMULATION

Cortical plasticity is defined as a property of central nervous system to reconfigure the structural and functional organization of central nervous in response to environmental and internal demands (Li et al., 2014). Neurogenesis, adding, removing, strengthening or weakening of synaptic connectivity are some vias to evaluate the plasticity (Pascual Leone et al., 2011; Freitas et al., 2013). Plasticity is the physiological basis for adaption of cognition, and behavior (Kuo et al., 2014). Experience is necessary in the phenomenon of plasticity induced by stimulation to cause changes in the brain organization, such as strength of connections, representational patterns and neuronal properties. Representational patterns might be modified for specific experiences. For

example, expert violin players have a larger right somatosensory cortex compared with non musician people (Schwenkreis et al., 2007). Neuronal properties might be altered, as is the case when electrical current stimulation is used. For instance, after anodal stimulation of the left primary motor cortex (Nitsche and Paulus, 2001), electroencephalogram recordings show an increase in the high gamma band (60-90 Hz) (Polania et al., 2011). Excitatory and inhibitory circuits are regulated by experience. Besides, neuronal plasticity may have a clear functional correlation, although sometimes correlation could be difficult to elucidate.

In early development, it can be found magnificent examples of brain plasticity: is known that infants (six months onward) begin to show preferences (frequency suction, head turning) for the phonemes of their native language on phonemes of foreign languages. One year old infants no longer respond to the phonemes of foreign languages; this could be due to the continuous contact with particular sounds of the native language that may promote the development of brain circuits associated with experienced sounds (Kuhl et al., 1992; Männel and Friederici, 2013).

The maturation of the visual cortex in non human mammals is another example of plasticity. The correct development of visual cortex is determined by visual experience during a critical period. Depriving animals of visual experience during a specific time period in early age disturbs the development of brain circuits and the function of the visual cortex (Hubel and Wiesel, 1965; 1970; 1980). These research provide evidence that the brain translates the effects of experience into a normal development brain circuitry or an altered brain circuitry. To understand how the experience changes neural circuits is necessary to access to molecular levels, because surely there is a base on signals generated by the synaptic activity associated with

sensory experience or motor performance. A receptor related to the synaptic plasticity in learning process is the glutamatergic ionotropic receptor NMDA (Hasan et al., 2013).

Non-invasive brain stimulation techniques are capable of inducing short-lasting plasticity in the brain (Ziemann et al., 2008). Transcranial magnetic stimulation and transcranial direct current stimulation are the two main non-invasive brain stimulation techniques to research about physiological brain functions, and also to explore therapeutic effects in psychological, psychiatric and neurological disorders, and for development and improvement of cognitive abilities.

Interest in non-invasive brain stimulation procedures is due to the opportunity of researching neuroplasticity in human brain. On the other hand, these tools keep the possible therapeutic potential in neurological, psychiatric and neuropsychological diseases.

1.3 TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation is a well-established non-invasive brain stimulation tool to activate cortical neurons via induction of a short-lasting strong electrical fields (Chen, 2000; Pascual-Leone, 2002; Pascual-Leone and Tormos-Muñoz, 2008; Nitsche and Paulus, 2009). This technique is based on the principles of Faraday´s electromagnetic induction in the XIX century. Nevertheless, it was in 1984 when Barker and collaborators developed the technique of the transcranial magnetic stimulation.

In general, transcranial magnetic stimulation is used as a research tool in order to analyze the brain processing. Furthermore, it is used in different clinical conditions (Medina and Túnez, 2013), specially in the treatment of major depression, being

approved by Food and Drugs Administration (FDA) in the Unites States of America. New expectations of treatment are being investigated in different medical and psychiatric illnesses such as Parkinson´s disease, Alzheimer dementia, motor rehabilitation in stroke patients, autism and schizophrenia (Bartrés-Faz et al., 2000; Pascual-Leone, 2006).

Transcranial magnetic stimulation generates an electric current; by a coil this current induces a magnetic field which, through the skull, can depolarize neurons inducing an electrical current in the brain. In the motor cortex, this current generate a *suprathreshold* motor cortex activation resulting in contralateral movements or muscle twitches by activating corticospinal tract, which serve as an index of motor cortex excitability (Kujirai et al., 1993; Rothwell, 1993; Koch et al., 2007). This tool stimulates excitable structures similarly to current injected using implanted or surface electrodes. Specific stimulation protocols are suited to evaluate the function of neuronal subgroups in the motor cortex (Ziemann et al., 2008), as well as cortico-cortical interactions.

1.3.1 Single-pulse transcranial magnetic stimulation

From the early uses of the transcranial magnetic stimulation, diverse protocols have been developed in order to study the neuroplastic mechanisms. One of the protocols more used is the single-pulse transcranial magnetic stimulation to study the motor cortex activity. The most usual coil is the eight shape coil (Figure 3). Every single pulse applied over the left primary motor cortex induces a depolarization and elicits a motor evoked potential in the contralateral hand. The amplitude of this induced potential can be registered and measured.

Subject is seated in a comfortable chair; to record the electrical activity of the hand two electrodes are placed in the right hand. The most usual muscles to place the electrodes are digiti minimi and interosseous muscles. Figure 4 shows the placement of the recording electrode over the interosseous muscle and the reference electrode over the second metacarpoghalangeal index finger. After that, the individual threshold is determined to provoke motor evoked potentials, and the amplitude of each one can be verified in the screen of the computer.

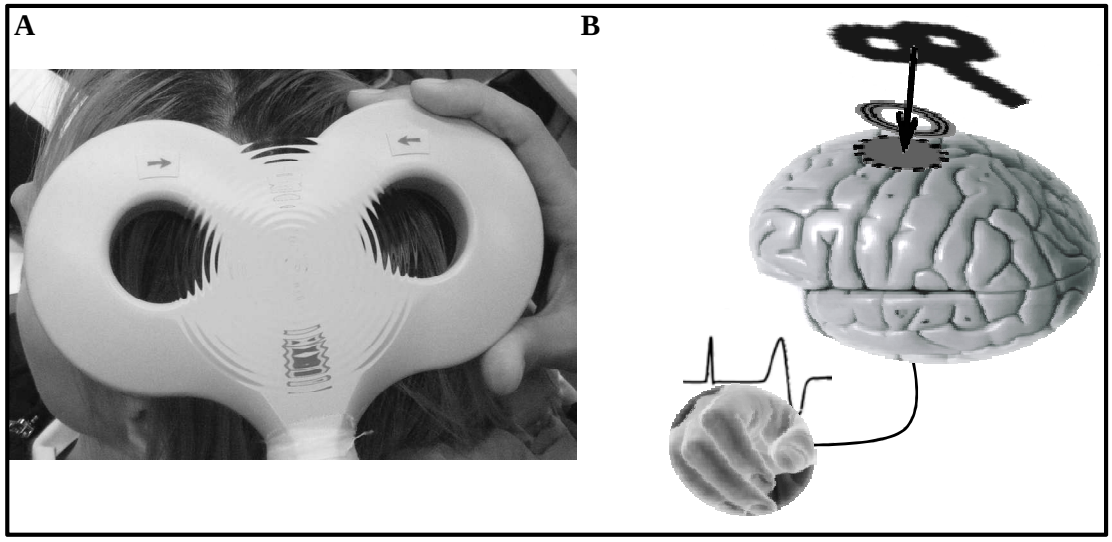


Figure 3. Transcranial magnetic stimulation coil. A standard eight shape coil is placed over the motor cortex through the skull. The magnetic pulse starts from the intersection of the two circles forming the coil (**A**). The pulse might depolarize motor cortex, and then the evoked potential can be registered (**B**).

Transcranial magnetic stimulation can be used to measure the motor cortex plasticity. For example, it has been proposed that the motor cortex excitability changes across the lifespan could be measured by single-pulse transcranial magnetic stimulation

(Freitas et al., 2013). In a recent study, Pisoni et al. (2014) found increases in the motor cortex excitability when subjects participating in two economic games. Single-pulse transcranial magnetic stimulation was applied in both conditions, but the excitability increases were only observed if the status of the game was changed so that the subject won or lost money. This effect was considered by authors as a motor facilitation induced by this technique related to economic reinforcement.

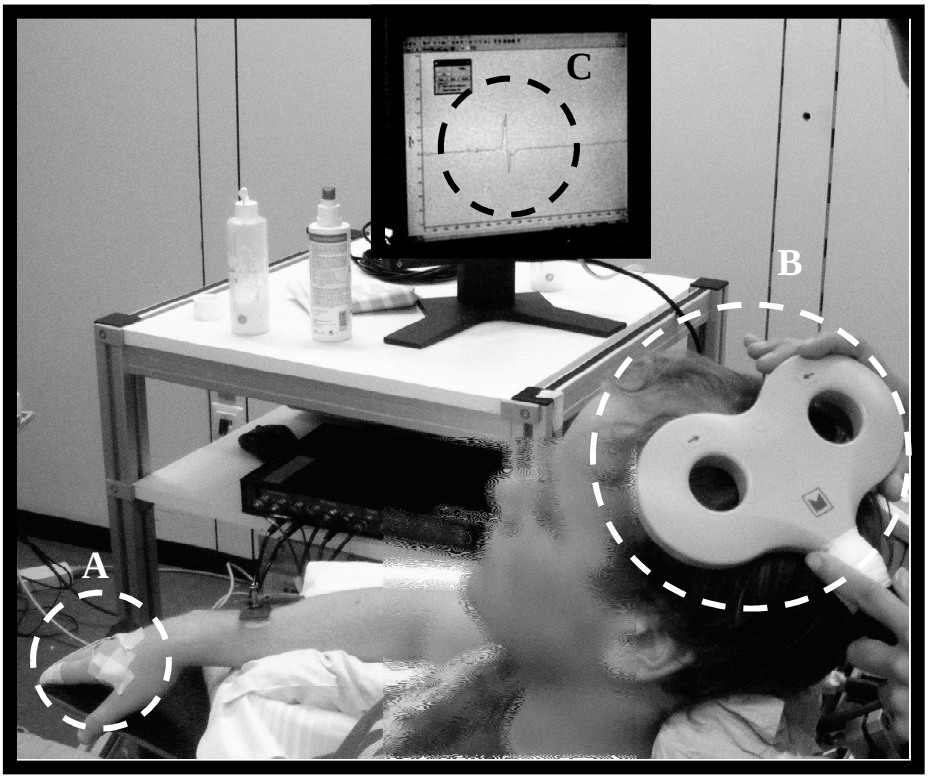


Figure 4. Transcranial magnetic stimulation setting. Subject is seated in a comfortable reclining chair with a mounted headrest. Two electrodes are placed: the recording electrode over the interosseus muscle, and the reference electrode over the index finger (**A**). The standard figure eight coil is placed over primary motor cortex (M1) (**B**); every single pulse can evoke a motor evoked potential (MEP). The amplitude of every MEP can be observed and measured in the screen of the computer (**C**).

Transcranial magnetic stimulation intensity is a threshold level of excitability, and motor thresholds are the minimum intensity to elicit a motor evoked potential.

Resting motor threshold (Figure 5A) is defined as the lowest stimulus intensity that elicits a peak-to-peak motor evoked potential amplitude of 50 mV or more in the resting muscle in at least three out of six trials. Active motor threshold (Figure 5B) is defined as the lowest intensity to evoke a motor evoked potential of higher amplitude compared to the muscular background activity in at least three out of six trials (Rothwell et al., 1999; Nitsche et al., 2005).

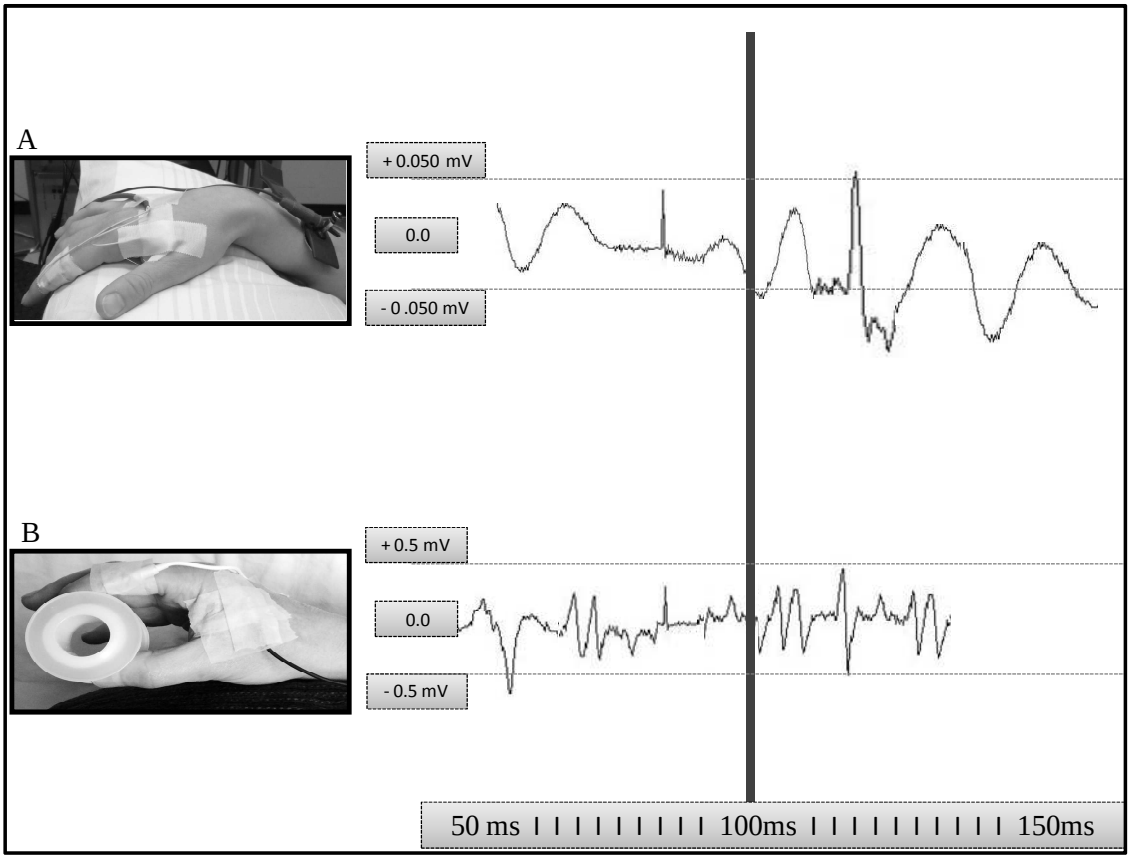


Figure 5. Resting motor threshold (RMT) (A) and active motor threshold (AMT) (B) setting. For RMT the hand must be relaxed and the amplitude signal should be (+/-) 0.050 mV. RMT is determined by the minimum intensity for which three out of six motor evoked potentials can be evoked (**A**). AMT is determined by the minimum intensity for which three out of six motor evoked potentials can be evoked at the time when the first dorsal interosseous muscle is activaded with an amplitude signal (+/-) of 0.5 mV (**B**).

Input-Output curve is an index of motor cortex excitability. In contrast with motor thresholds, it is an index of a larger neuronal population involved. Input-output curve is recruited eliciting blocks of motor evoked potentials by a single pulse, increasing the intensity over the 100% of resting motor threshold (Nitsche et al., 2005).

Some research has shown that the slope of the input-output curve is decreased in amyotrophic lateral sclerosis (Khedr et al., 2011). In other study, smokers participants increase the motor cortex excitability in this curve at 150% of resting motor threshold compared to non smokers (Grundey et al., 2013), but this difference in the motor cortex excitability could not be registered in smokers using another intensities (Lang et al., 2008; Grundey et al., 2013).

1.3.2 Paired-pulse transcranial magnetic stimulation

Transcranial magnetic stimulation can be used applying double pulse (as conditioning and test stimuli) in a short period of time through one coil. This paired-pulse transcranial magnetic stimulation is used to induce facilitation and inhibition in the motor cortex. The first pulse is a conditioning stimulus which is applied before the transcranial magnetic stimulation or test stimulus. Depending on the time proximity between both stimuli presentation, it can be activated a pathway of intracortical inhibitory neurons, or a pathway of intracortical facilitatory neurons.

The interstimulus intervals associated with inhibitory pathways are 2, 3, 5 and 7 ms (Figure 6). Depending on the time interval in which the conditioning stimulus is applied before the test pulse, the answer to this stimulation changes because the closer the conditioning stimulus is to the test pulse, the more inhibited is the motor evoked

potential. Interstimulus intervals of 10 and 15 ms (Figure 7) are related to facilitatory pathways (Kujirai et al., 1993; Ziemann et al., 1996; Rothwell, 1997). In this case, a 15 ms interstimulus interval produces bigger facilitation of the motor evoked potentials than a 10 ms one.

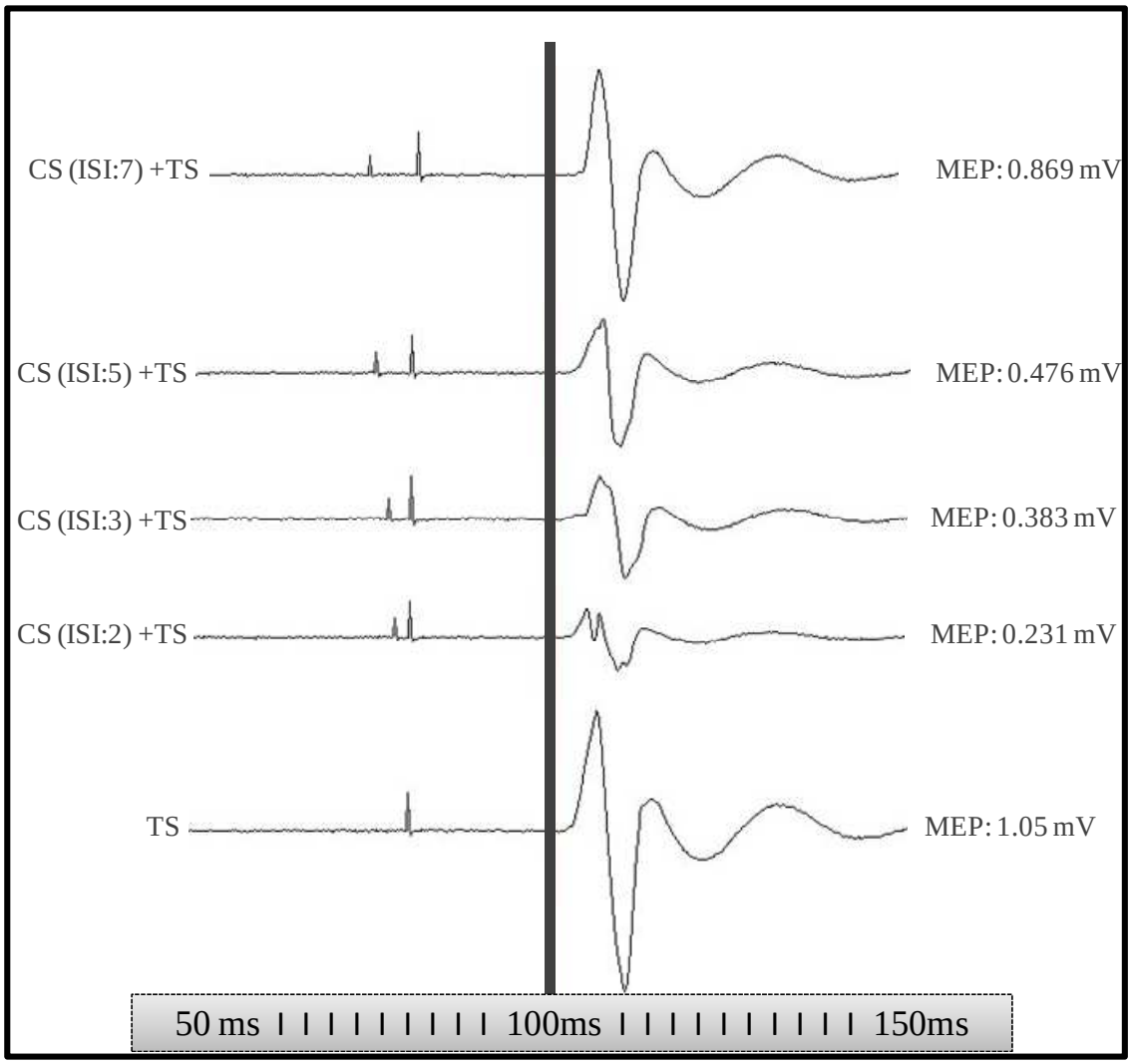


Figure 6. Short intracortical inhibition (SICI). Test stimulus (TS) is one pulse given by transcranial magnetic stimulation to evoke a motor evoked potential (MEP). Conditioning stimulus (CS) is a pulse applied before the TS. CS are applied at different interstimulus intervals (ISIs) of 2, 3, 5 and 7 ms. The motor evoked potential inhibition magnitude depends on the time interval between the CS and the TS.

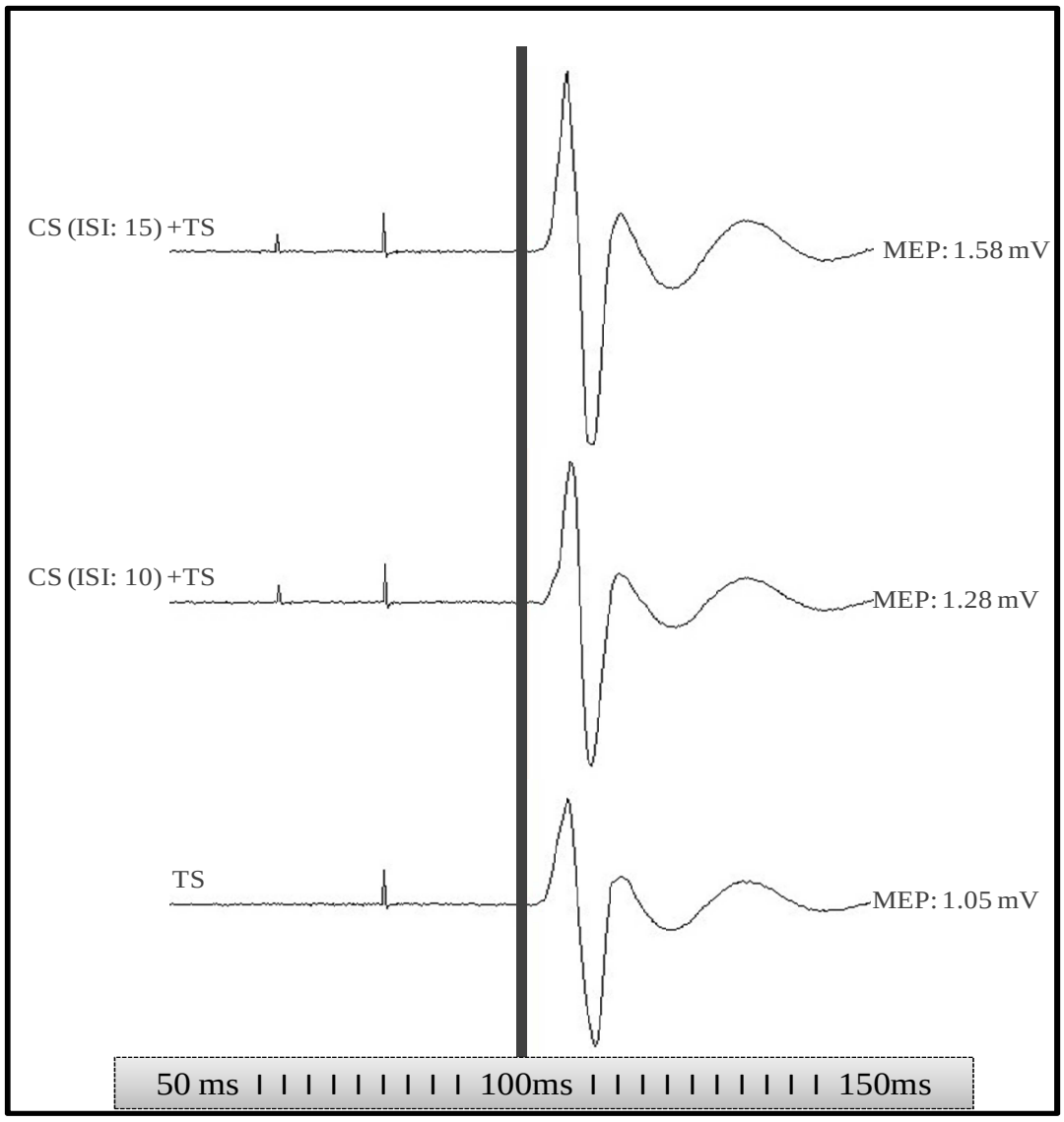


Figure 7. Intracortical facilitation (ICF). Depending on the time interval in which the conditioning stimulus (CS) is applied before the test stimulus (TS), the motor evoked potential (MEP) amplitude changes. With interstimulus intervals (ISIs) of 10 ms and 15 ms, the MEP amplitude enhances.

The intensity of the test stimulus is determined for the threshold to evoke a motor evoked potential of 1 mV peak to peak amplitude. This is the single test pulse for evoking motor evoked potentials. Conditioning stimulus intensity depends on the specific protocol and it is determined for the intensity to evoke the resting motor threshold or the active motor threshold. To avoid any floor or ceiling effect, the

intensity of the conditioning stimulus is set to a lower value to the 100 % of active o resting motor thresholds (Nitsche et al., 2005).

Intracortical inhibition and facilitation in the motor cortex can differ between ages. Children show significantly less intracortical inhibition (1, 3 and 5 ms) compared to adults, and also adolescents show significantly less inhibition (5 ms) compared to adults (Walther et al., 2009). Motor cortex maturation could be guided by inhibition. The most accurate movements need a high inhibitory control of different muscles; writing is an example.

Paired pulses can be applied by two coils in order to research the connectivity between two areas in the brain. Twin coil transcranial magnetic stimulation (Figure 8) has proved to be dependable to study interactions between cortical areas (Koch, 2011). Ferbert et al. (1992) devised a paired-pulse transcranial magnetic stimulation protocol with two coils to investigate the inter-hemispheric connections. They placed the coil to induce the conditioning stimulus on the right motor cortex and the coil to apply the transcranial magnetic stimulation or test stimulus on the left motor cortex. When the conditioning stimulus was applied 7, 8, 9 and 10 ms before test stimulus, the amplitude of the motor evoked potential was reduced. Interestingly, this inhibition has not been found in patients with no corpus callosum (Meyer et al., 1995).

The specific connectivity between the premotor and primary motor cortex has been explored by twin coil transcranial magnetic stimulation. When a conditioning stimulus is applied around 6 ms over the left premotor cortex, then the motor evoked potential in the left primary motor cortex is reduced (Civardi et al., 2001). On the other hand, also the connectivity between parietal cortex and motor cortex has been studied using a twin coil transcranial magnetic stimulation protocol. Facilitatory pathways can

be activated when a conditioning stimulus is applied over posterior parietal cortex and the test stimulus is placed over the ipsilateral motor cortex (Koch et al., 2007, 2008).

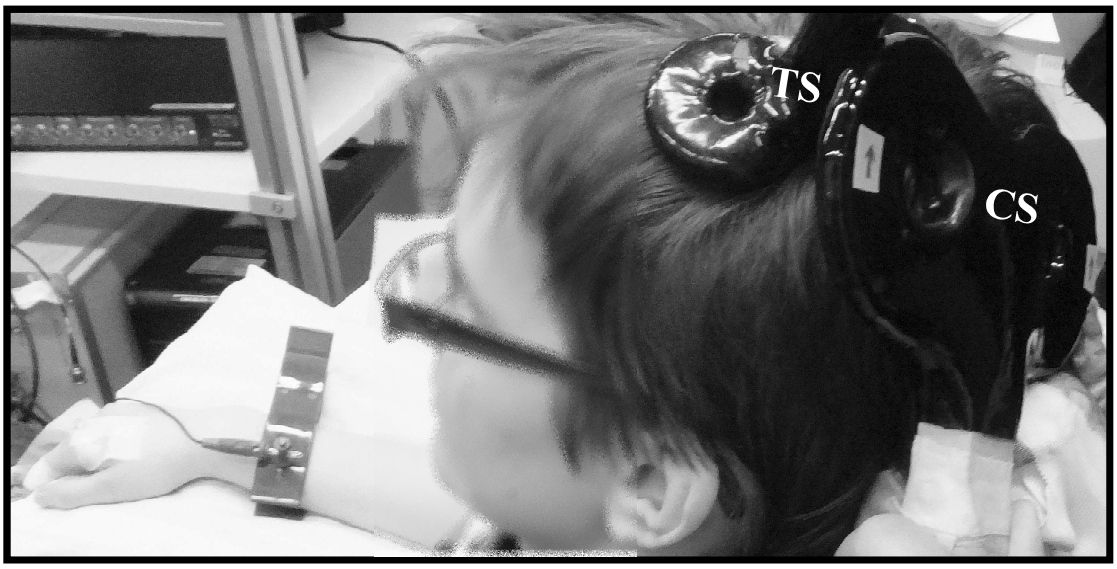


Figure 8. Twin coil transcranial magnetic stimulation protocol to study cortical connections. In this paradigm, each coil is placed in a specific area. For example, a conditioning stimulus (CS) applied in parietal cortex could activate pathways to the motor cortex. The second coil placed in primary motor cortex would produce the second trial (a few ms later), and this test stimulus (TS) would evidence any change in the motor cortex excitability produced by the CS.

Some evidence shows that motor cortex excitability measured by transcranial magnetic stimulation might be modified by specific auditory (Flöel et al., 2003; Sowman et al., 2014) or visual stimuli (Fadiga et al., 1995, 2005; Mattiassi et al., 2014). Moreover, motor evoked potentials and intracortical inhibition (Patuzzo et al., 2003) of the motor cortex are increased when a hand movement is observed, but there is no modification in the excitability of motor cortex when an object is observed (Fadiga et al., 1995; Patuzzo et al., 2003) or in a dimming detection condition (Fadiga et al., 1995). Motor evoked potentials amplitude of the motor cortex also increases when people

observe negative pictures (from the International Affective Picture System database). This change of excitability could be explained because negative emotions might require motor reactions, more than positive or neutral emotions (Borgomaneri et al., 2013).

Motor cortex excitability might also be modified through lifespan and by learning process (Walther et al., 2009; Freitas et al., 2013; Bashir et al., 2014). Some changes in the intracortical excitability through lifespan are related with the intracortical inhibition. These changes are less in children and adolescents compared with adults (Walther et al., 2009). On the other hand, intracortical inhibition is also desinhibited during motor learning (Coxon et al., 2014).

In summary, voluntary movements require an intricate interaction among cortical areas; in that sense, transcranial magnetic stimulation is a reliable tool to investigate this communicative network. Particularly, double-pulse transcranial magnetic stimulation provides the possibility to research inhibitory and facilitatory pathways.

1.4 TRANSCRANIAL DIRECT CURRENT STIMULATION

Transcranial direct current stimulation is a non-invasive and safety technique to stimulate specific areas of the brain. The pioneering experiments using electrical currents were done long time ago, at the end of the XVII century, by Galvani and Volta who formed the basis of the electrophysiology. Early XVIII century, Aldini (Galvani´s nephew) was the first person to use electrical currents over the head as a treatment for depression. The labor of these researchers was the basis for the development of many actual medical applications (Parent, 2004). In the thirties of the last century, Cerletti and

Bini (Aruta, 2011) introduced electroshock as a treatment for psychosis. Cerletti and Bini were nominated for Nobel Prize in several times in the early fifties for the use of the electroconvulsive therapy to treat psychotic disorders as schizophrenia or manic-depressive disorder. In the sixties, a new discovery about electrical current regained the interest for this technique again. Albert (1966) observed that the polarization of the electrical currents (anodal and cathodal) affects the consolidation of learning in rats. In the 90's emerged a new treatment to lessen the symptoms of Parkinson's disease: the deep brain stimulation surgery, which produced benefits in the reduction of motor symptoms of this disease (Caparros-Lefebvre et al., 1993; Krauss and Jankovic, 1996). Priori et al. (1998) were the first to report changes in the human motor cortex excitability after transcranial direct current stimulation. Specifically, they observed a relation between cathodal stimulation and increases in the motor cortex excitability. Subsequently, Nitsche and Paulus (2000) found that anodal transcranial direct current stimulation applied over the motor cortex enhances the motor cortex excitability and cathodal current decreases the excitability. Since these studies, many researchers have been interested in the use of transcranial direct current stimulation as a useful tool for neuroscience.

Transcranial direct current stimulation in humans is induced by low frequency electric current. The density of the current depends on the size of the electrodes. Density typically varies between 0.029 and 0.08 mA/cm^2 (Paulus et al., 2012). A standard electrode size is 35 cm^2 (Nitsche and Paulus, 2000). The lowest intensity to induce excitability changes in the motor cortex is 0.4 mA, and a safety frequency up to 2.0 mA (Nitsche et al., 2005). A 3.0 mA intensity is already a painful stimulus (Furubayashi et al., 2008). Electrodes size from 35 cm^2 to 16 cm^2 are related with less cutaneous

sensation (itching, tingling) during the stimulation (Turi et al., 2014). Regarding the time of stimulation, at least 3 min of stimulation is necessary to change the motor cortex excitability (Nitsche and Paulus, 2000).

Certain brain stimulation protocols by transcranial direct current stimulation enable the induction of plasticity via the application of weak direct current through the scalp (Pascual-Leone et al., 1999; Nitsche and Paulus, 2000; Nitsche, 2002; Nitsche et al., 2002; Lang et al., 2004; Priori et al., 2009; Polanía et al., 2011; Stagg and Nitsche, 2011). Dependent on the polarity of stimulation, long-lasting motor cortex excitability enhancements or reductions are induced, which depend on the glutamatergic neurotransmission system (Nitsche et al., 2003a,b,d, 2005; Nitsche and Paulus, 2011). Anodal and cathodal are the two kinds of currents used in the transcranial direct current stimulation technique. It has been shown that anodal current stimulation increases the cortical excitability, meanwhile cathodal current stimulation reduces it (Nitsche et al., 2005). For research purposes, sham is a third kind of stimulation which consists of a false stimulation used to compare the after effects of anodal and cathodal transcranial direct current stimulation. Besides polarity, intensity is other important variable for transcranial direct current stimulation. The intensity range to stimulate the brain can produce different physiological effects. Cathodal transcranial direct current stimulation at 2 mA results in significant increased of primary motor cortex excitability (Batsikadze et al., 2013). For motor learning, anodal transcranial direct current stimulation at 1.5 mA has proved a significant improvement of motor learning curve, but 1mA anodal tDCS do not result in the same significant improvement effect (Cuypers et al., 2013).

Transcranial direct current stimulation has proved to be a good tool to evoke neuroplasticity in animals (Molaee-Ardekani et al., 2013) and humans. Some

researchers have focused to look for different applications of this tool in the human brain, as the study of functional correlation of the stimulation or possible improvement of the cognitive functions. In this regard, it has been shown that transcranial direct current stimulation over left dorsolateral prefrontal cortex improves working memory in healthy participants (Fregni et al., 2005b), acts as a modulator of verbal fluency networks in Parkinson disease (Pereira et al., 2013) and reduces the perceived degree of negative emotions (Peña-Gómez et al., 2011). Moreover, a better execution of the performance hand and motor learning has been reported by anodal transcranial direct current stimulation applied over the left primary motor cortex (Nitsche et al., 2003d; Pavlova et al., 2014). In clinics, it has been a useful technique for defining that the recovery of function after a stroke is determined by the neural network involving the affected and the unaffected brain hemisphere. Excessive activity in the unaffected hemisphere may represent a maladaptive strategy. In this sense, cathodal stimulation of the unaffected hemisphere and anodal stimulation of the affected hemisphere might improve motor performance significantly (Fregni et al., 2005a). Finally, transcranial direct current stimulation has also been explored to reduce the perception of pain in patients with neuropathies (Kumru et al., 2013).

1.5 NON-INVASIVE BRAIN STIMULATION AND PRIMARY MOTOR CORTEX PLASTICITY

Learning and memory are produced through different neurophysiological mechanisms that involve several changes in specific synaptic connections. These changes in the effectiveness of certain connections between neurons, associated with learning and memory, include molecular, electrochemical and structural modifications.

The ability of brain cells to alter their operation by the action of certain stimuli is known as plasticity, and in this process are described two phenomena: the long-term potentiation and the long-term depression. Brain plastic changes have been studied and demonstrated in different animal models (Malenka and Bear, 2004). In humans, brain plasticity has also been studied in different ways (Pascual-Leone et al., 1999; Nitsche et al., 2004, 2007; Ziemann et al., 2008). Neurophysiological recording and neuroimaging techniques have been useful to elucidate the plastic processes necessary for learning and memory in humans. More specifically, brain stimulation techniques are providing fundamental information nowadays to understand the neurophysiological mechanisms of brain plasticity associated with learning (Nitsche et al., 2010). Learning-related plasticity is often explored as a local process (Taubert et al., 2011b), but alterations of cellular activity during learning are presumed to take place at functionally interconnected areas (Chen et al., 2003; Koch et al., 2008; Feurra et al., 2011; Taubert et al., 2011a).

Although the plasticity mechanisms associated with learning are developed in specific regions of the cerebral cortex, some inter-regional mechanisms may also be necessary. Thus, learning in general requires regional plastic changes, whose location depends on the modality of learning and the type of task (Honda et al., 1998), but also depends on cortico-cortical connectivity. The plasticity of these connected areas is being explored nowadays intensively in order to elucidate its specific relationship with learning.

In this sense, transcranial direct current stimulation is well suited to explore the plasticity of interregional cortical connectivity, as shown by the ability to induce plasticity of premotor-motor cortex connections (Boros et al., 2008). On the other hand,

has also been shown that this tool improves motor learning (Nitsche et al., 2003c; Antal et al., 2004; Reis et al., 2009; Nitsche et al., 2010). Intracortical facilitation and intracortical inhibition of the primary motor cortex can be modified by anodal transcranial direct current stimulation over premotor cortex. Intracortical inhibition is reduced in the interstimulus interval of 2 and 3 ms, and intracortical facilitation was enhanced at the interstimulus interval of 10 and 15 ms. However, cathodal current stimulation applied on the left premotor cortex induced no change in the excitability of primary motor cortex (Boros et al., 2008). As mentioned previously, the results of the studies with anodal and cathodal transcranial direct current stimulation are usually compared to those obtained by a false stimulation (sham) to form a control group.

In rabbits, it has been shown that anodal current can improve and cathodal current can depress the subjective sensation of sensory stimuli used during a pavlovian conditioning test (Márquez-Ruiz et al., 2012). Interestingly, the depressing effects evoked by cathodal stimulation seem to be mediated by adenosine receptors located in cerebral cortex circuits (Márquez-Ruiz et al., 2012).

1.5.1 Plasticity of interregional connections of the primary motor cortex

Interregional mechanisms are thought to be determined by the functional connectivity between two or more areas involved in each learning process. In motor learning, interregional connectivity involves the primary motor cortex, supplementary motor cortex and dorsal premotor cortex (Frey and Gerry, 2006; Vahdat et al., 2011). Some studies have shown the possibility to induce interregional plasticity in interconnected areas, specifically in relation to the motor cortex (Boros et al., 2008). In that research,

sixteen subjects received anodal and cathodal transcranial direct current stimulation over premotor cortex, and 8 subjects over the dorsolateral prefrontal cortex. Motor cortex excitability was monitored by short-latency intracortical inhibition, intracortical facilitation and other typical protocols. Interestingly they found a selective effect, since premotor anodal transcranial direct current stimulation decreased the short-latency intracortical inhibition and increased the intracortical facilitation. This selective influence of anodal stimulation over premotor cortex was considered by the authors as a connectivity effect between premotor cortex and primary motor cortex.

1.5.2 Primary motor and parietal cortices connectivity and functional relevance

Different association areas related to motor responses, such as parietal cortices, may have a functional connectivity with the motor areas significant for motor learning, which could be reflected in a learning-related reorganization of the respective network. In this regard, the involvement of the parietal cortex in the motor cortex activity has been studied in humans by neuroimaging (Pascual-Leone et al., 2011) and brain stimulation (Koch et al., 2007, 2008; Veniero et al., 2013). Using paired-pulse transcranial magnetic stimulation over the parietal cortex by twin coils and applying the conditioning stimulus at 90% of the resting motor threshold, Koch et al. (2007) have suggested that there are facilitatory connections between the caudal part of the inferior parietal sulcus site and ipsilateral motor cortex, mediated by specific interneurons in the motor cortex. The authors propose that these connections could be important for motor learning.

In a recent research, Chao et al. (2013) applied corticocortical paired-associative stimuli to the ipsilateral posterior parietal cortex and primary motor cortex. By this procedure, they found that parietal stimulation at the interstimulus interval of 8 ms increases the excitability of conditioned left primary motor cortex assessed by motor evoked potentials and the input-output curve. Thus, transcranial magnetic stimulation pulses over parietal cortex can modulate primary motor cortex and posterior parietal cortex to primary motor cortex pathway excitabilities. The authors conclude that this could be a new approach to modify motor cortex excitability and sensorimotor interaction.

Chao et al. (2013) also found that paired-associative stimuli applied to the posterior parietal cortex do not induce changes in the motor behavior assessed by the *Purdue pegboard task.* Nevertheless, recent studies have shown that transcranial direct current stimulation applied over P3 can modify the performance in motor tasks. Convento et al. (2014) have found that anodal current stimulation (2 mA, 10 min) over the left posterior parietal cortex selectively facilitates action planning, while the anodal current stimulation over the right primary motor cortex only modulates the action execution. This evidence shows that motor abilities relay on substantial different mechanisms. Other studies have also shown this relationship between cortical stimulation by transcranial direct current stimulation and the results in functional tasks. For example, Medina et al. (2013) applied anodal, cathodal or sham current stimulation over P3 using two electrodes of size 25 cm^2 (5×5 cm). The anodal electrode was located over the left parietal cortex, and the cathodal electrode was located over the right parietal cortex (P4), and vice versa. Transcranial direct current stimulation was applied at 1.5 mA for 20 minutes. They used a target detection task in which the target – a circle

with a gap - was either to the right or left of the viewer (egocentric condition), or contained a gap on the right or left side of the circle (allocentric condition). Subjects performed the task before, during, and after transcranial direct current stimulation. The results indicated that the right anodal-left cathodal transcranial direct current stimulation has a facilitatory effect on allocentric visuospatial processing.

Finally, transcranial direct current stimulation has been applied to improve some symptoms in different clinic conditions. For instance, since a limb amputation might induce maladaptative neuroplasticity, Bolognini et al. (2013) applied stimulation to the primary motor cortex and P3 to evaluate the effects on the phantom limb pain and sensations. They found that the painful and non-painful phantom limb sensations are dissociable phenomena. Non-painful phantom sensations are associated to a hyperexcitation of posterior parietal cortex that could be normalized by cathodal transcranial direct current stimulation (2 mA, 15 min) over P3. Phantom limb pain is associated with cortical excitability in the sensorimotor network; increasing excitability in this system by anodal transcranial direct current stimulation (2 mA, 15 min) over primary motor cortex they observed an analgesic effect on phantom limb pain. The results of this study indicate that the level of excitability of different cortical areas influences the painful and non painful sensations in phantom limb, and the modulation of this excitability by transcranial direct current stimulation can reduce these symptoms. In addition to this potential therapeutic use, the effects of transcranial direct current stimulation have been studied in the context of other different pathologies, like neglect (Medina et al., 2013) or motor and apraxic disorders in patients with stroke (Convento et al., 2014).

2. OBJECTIVES OF THE RESEARCH

In humans, the anterior parietal cortex is a primary area for processing sensory information, whereas posterior regions of the parietal cortex appear to be involved in the integration of sensory-motor activities (Krause et al., 2012). In particular, the posterior parietal cortex is related to motor learning (Shum et al., 2011). Performance of motor tasks enhances the activity of this area (Honda et al., 1998). Specifically, connections between the parietal, primary motor and premotor cortex are thought to convey information relevant for planning of movements in space. Connections between the parietal and motor cortex are functionally enhanced during early stages of planning of reaching movements (Koch et al., 2008).

Alterations of cellular activity during learning, especially in the form of long term potentiation (LTP) (Malenka and Bear, 2004; Nitsche et al., 2003a; Rioult-Pedotti et al., 1998, 2000; Ziemann et al., 2008), have been observed at topographically distant, but functionally interconnected, areas (Chen et al., 2003; Koch et al., 2008). In motor learning, interregional connectivity involves a distributed cortical network including the primary motor cortex, supplementary motor area, premotor and parietal cortices (Vahdat et al., 2011). Direct physiological effects of acute parietal cortex activation on primary motor cortex excitability and activity have been reported (Koch et al., 2007, 2008). Specifically, facilitatory connections between the caudal part of the inferior parietal sulcus and the ipsilateral motor cortex have been identified by transcranial magnetic stimulation (Karabanov et al., 2013, Koch et al., 2007, 2013). Nevertheless, the plasticity of these interregional connections, which might have functional implications for motor learning, awaits to be elucidated.

Here we explore the impact of plasticity induction of the parietal cortex by transcranial direct current stimulation on excitability of the ipsilateral primary motor

cortex in healthy humans, in order to learn more about plasticity of these functionally interconnected areas. Transcranial direct current stimulation is a non-invasive brain stimulation tool which enables the induction of plasticity via application of weak direct currents through the scalp (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2002, 2003b, 2008; Priori et al., 2009; Stagg and Nitsche, 2011). The primary effect is a polarity-dependent shift of resting membrane potentials, and sufficiently long stimulation results in long-lasting excitability enhancements or reductions, which depend on the glutamatergic and GABAergic systems (Nitsche and Paulus, 2001; Nitsche et al., 2003b; 2008; Nitsche and Paulus, 2011; Nitsche et al., 2005). Transcranial direct current stimulation is suited to explore plasticity of interregional cortical connectivity, as shown by its ability to induce plasticity of premotor-motor cortex connections (Boros et al., 2008), and has been shown to improve motor learning (Nitsche et al., 2003c; Reis et al., 2009). We hypothesized that excitability-enhancing anodal transcranial direct current stimulation applied to the posterior parietal cortex region (P3) of the international 10-20 electroencephalography system will enhance primary motor cortex excitability, while cathodal transcranial direct current stimulation over the same area will result in antagonistic effects.

Transcranial direct current stimulation is a useful tool to study the plasticity of the human brain cortex and its relation to different neurological disorders. More recently, non-invasive brain stimulation, and in particular the transcranial direct current stimulation, are being used to understand the cortico-cortical plasticity process. Transcranial magnetic stimulation and transcranial direct current stimulation are non-invasive brain stimulation techniques that can be used to explore both, regional and interregional mechanisms of plasticity and its relationship to learning (Nitsche and

Paulus, 2011). But the specific plasticity of the parietal-motor connectivity and the functional importance of the respective plastic alteration for motor learning have not been explored systematically so far, and this is the general objective of this thesis.

In summary, the main objectives addressed in this doctoral thesis are:

1. To explore the changes in primary motor cortex excitability after anodal, cathodal or sham transcranial direct current stimulation applied over P3.

2. To explore the effects on primary motor cortex related to spatial specificity after anodal or cathodal transcranial direct current stimulation applied over cortical areas adjacent to P3 (3 cm lateral or 3 cm posterior to P3).

3. To research the changes in the short intracortical inhibition and intracortical facilitation induced by anodal or cathodal transcranial direct current stimulation over P3.

4. To study the alterations of parieto-motor connectivity elicited by anodal or cathodal transcranial direct current stimulation over P3.

3. MATERIALS AND METHODS

3.1 SUBJECTS

Thirty seven right-handed healthy subjects, 17 men and 20 women, aged 20 to 54 years (mean age = 28.6 ± 8.0 years), participated in this project. Fourteen of them, 7 men and 7 women, aged 20 to 48 years (mean age = 28.3 ± 9.4 years), participated in experiment 1a. Thirteen subjects, 7 men and 6 women, aged 24 to 54 years (mean age = 28.6 ± 8.0 years), participated in experiment 1b (two of whom also participated in the previous one). In the last two experiments (2a,b), fifteen subjects (three of whom had taken part in the second experiment), 5 men and 10 women, aged 19 to 31 years (mean age= 25.7 ± 3.4 years) were included. None of the participants was taking medication, and none reported previous or present neurological or psychiatric diseases. All subjects gave informed written consent before participation and were compensated for participation. The study was approved by the Ethics Committee of the University of Göttingen, and conforms to the Declaration of Helsinki.

3.2 PLASTICITY INDUCTION BY TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

Transcranial direct current stimulation (tDCS) was performed by battery-driven constant-current stimulators (NeuroConn GmbH, Ilmenau, Germany/Starstim Neuroelectrics, Barcelona, Spain) with conductive rubber electrodes, placed between two saline-soaked sponges. The electrode size used for parietal transcranial direct current stimulation was 15 cm^2 (3x5 cm). The return electrode size was 35 cm^2 (7x5 cm). The return electrode was placed over the right supraorbital ridge. To stimulate the left parietal cortex, the respective electrode was placed over the posterior parietal cortex

(P3) region (Figure 9) according to the 10-20 electroencephalography international system (Herwig et al., 2003), as well as 3 cm lateral or posterior to P3 in single experiments. The electrodes were fixed onto the head by elastic rubber bands.

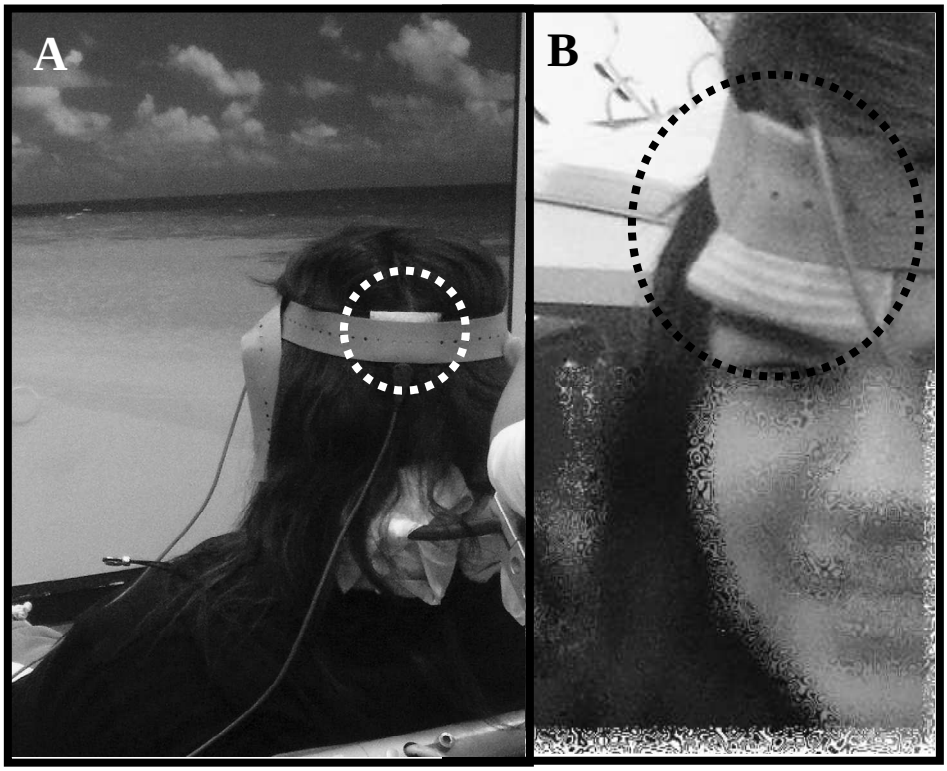


Figure 9. Transcranial direct current stimulation (tDCS) electrodes setting. Electrodes were placed according to the 10-20 electroencephalography international system and fixed onto the head by elastic rubber bands. (**A**) Subjects were seated in chair with a mounted headrest during the experiment. To stimulate the left parietal cortex, the active electrode was placed over the posterior parietal cortex region (P3) (white dotted circle); the electrode size used for parietal tDCS was 15 cm^2 (3x5 cm). (**B**) The return electrode size was 35 cm^2 (7x5 cm), was placed over the right supraorbital ridge (black dotted circle).

Transcranial direct current stimulation was performed for 15 min, because similar conditions result in excitability changes stable for about 1 hour after motor cortex stimulation (Nitsche and Paulus, 2001; Nitsche et al., 2003c). It was applied with

a current strength of 0.5 mA with gradual increase and decrease for 7 seconds at the beginning and the end of stimulation, respectively. All subjects felt a mild tingling sensation under the active and return electrodes, which subsided during the first minutes. Subjects were blinded for transcranial direct current stimulation conditions. Each subject received stimulation of the left parietal cortex (P3), both anodal and cathodal, in randomized order and on separate days at least 1 week apart. For sham transcranial direct current stimulation, current was increased and then decreased over 8 sec both, at the beginning and end of a 15 min stimulation session, in order to ensure perception of some tingling sensation under the electrodes, but did not receive stimulation during the remaining session.

3.3 MONITORING OF MOTOR CORTEX EXCITABILITY BY TRANSCRANIAL MAGNETIC STIMULATION (TMS)

Transcranial Magnetic Stimulation was accomplished by a standard double of eight shaped 70-mm coil (Figure 3) connected to a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) for obtaining single-pulse TMS-elicited motor evoked potentials. The coil was placed tangentially to the scalp, with the handle pointing postero-laterally at a 45º angle from the midline. The optimal position was considered as the site where transcranial magnetic stimulation resulted consistently in the largest motor evoked potentials in the resting target muscle, the right first dorsal interosseus muscle. The representation of the primary motor cortex was marked on the scalp with a skin marker. Surface electromyography was recorded from the right first dorsal interosseus muscle by use of Ag–AgCl electrodes. The active electrode was placed over the first dorsal interosseus muscle belly, and the reference electrode over

the tendon of the this muscle. The signals were amplified and filtered (2 Hz to 2 kHz, sampling rate of 5 kHz), digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK), and recorded by computer software (SIGNAL, Cambridge Electronic Design, version 2.13) for off-line analyses.

Resting motor threshold was defined as the lowest stimulus intensity that elicited a peak-to-peak motor evoked potential amplitude of 50 μV or more in the resting muscle in at least three out of six recordings. Active motor threshold was considered as the minimum intensity eliciting an motor evoked potential of superior size in relation to moderate spontaneous muscular background activity (~15% of the maximum muscle strength) in at least three out of six trials (Nitsche et al., 2005).

For obtaining single test pulse motor evoked potentials, the transcranial magnetic stimulation intensity which resulted in an average of the motor evoked potentials amplitude of about 1 mV peak-to-peak before the stimulation was identified for baseline determination, and kept constant throughout the remaining experiment, unless adjusted in case of double stimulation protocols.

Input-output curve was determined using increasing stimulus intensities (100, 110, 130 and 150% of resting motor threshold), each 20 pulses per block (Nitsche et al., 2005).

Short interval intracortical inhibition and intracortical facilitation within the primary motor cortex were obtained by a paired-pulse transcranial magnetic stimulation protocol. The intensity of the conditioning stimulus was set to 70% of the active motor threshold, and the test stimulus was adjusted to the intensity to evoke a motor evoked potential of about 1 mV peak-to-peak amplitude. Interstimulus intervals between the

pairs of stimuli were: 2, 3, 5, 7, 10, and 15 ms. We pooled data for inhibitory (2-3 ms), neutral (5-7 ms), and facilitatory (10-15 ms) stimulation. The exact interval between the paired-pulses was randomized (4 ± 0.4 s). The pairs of stimuli were organized in randomized and mixed order in 13 blocks, in which each interstimulus interval was represented once and an additional single test pulse was applied. The mean peak-to-peak amplitude of the conditioned motor evoked potential at each interstimulus interval was expressed as a percentage of the mean peak-to peak size of the unconditioned test stimulus.

In the paired-pulse twin coil of the transcranial magnetic stimulation protocol, which was conducted to explore parieto-motor cortical connectivity, the test pulse was applied by a 50 mm figure-of-eight-shaped coil placed over the primary motor cortex representation of the right first dorsal interosseous. The conditioning stimulus was applied by a 70 mm eight-shaped coil placed over P3. Conditioning stimulus intensity was set to 90% of the resting motor threshold. Interstimulus intervals between the pairs of stimuli were 2, 4, 6, 8, 10, and 15 ms. We also pooled data for inhibitory (2-3 ms), neutral (5-7 ms), and facilitatory (10-15 ms) stimulation. The interval between the paired pulses was randomized (4 ± 0.4 s). The pairs of stimuli were organized in randomized and mixed order in 10 blocks, in which each interstimulus interval was represented once and the single test pulse was applied twice.

3.4 EXPERIMENTAL PROCEDURES

Subjects were seated in a comfortable reclining chair with a mounted headrest during the experiment. The electromyography electrodes were placed over the right first dorsal

interosseous muscle for obtaining motor evoked potentials. All subjects received transcranial direct current stimulation of 0.5 mA to the left posterior parietal cortex for 15 min, both anodal and cathodal in randomized order and on separate days at least 1 week apart to avoid carryover effects. Cortico-spinal and cortico-cortical excitability of the ipsilateral primary motor cortex were first monitored by transcranial magnetic stimulation to determine baseline measures. Then, transcranial direct current stimulation was applied as described above. After the stimulation, cortico-spinal and cortico-cortical excitability were monitored by transcranial magnetic stimulation with the same parameters obtained for baseline measures at different time intervals after stimulation.

3.4.1 Experiment 1a

Primary motor cortex excitability changes induced by parietal transcranial direct current stimulation

Fourteen right-handed healthy subjects, 7 men and 7 women, aged 20 to 48 years (mean age = 28.3 ± 9.4 years), participated in this experiment. We investigated the neuroplastic changes induced in primary motor cortex after applying transcranial direct current stimulation over the left posterior parietal cortex (P3). All subjects received anodal and cathodal transcranial direct current stimulation over the P3 position of the 10-20 electroencephalography international system, six of them also received sham stimulation. Transcranial direct current stimulation conditions were applied in randomized order and on separate days at least 1 week apart to avoid carryover effects. Before the stimulation was applied, cortico-spinal excitability was first monitored by determination of resting motor threshold and input-output curve as described above, and

by single test-pulse transcranial magnetic stimulation with a TMS-intensity resulting in ~1mV peak-to-peak amplitude of the motor evoked potential of the right first dorsal interosseous. For single-pulse transcranial magnetic stimulation, 20 motor evoked potentials were obtained for baseline determination (frequency of 0.25 Hz +/- 10%), and the transcranial magnetic stimulation intensity was kept constant for the remaining experiment. Then transcranial direct current stimulation was applied as described above. After transcranial direct current stimulation, cortico-spinal excitability was monitored by transcranial magnetic stimulation with the parameters obtained for baseline measures, 30 min and 60 min later (Figure 10).

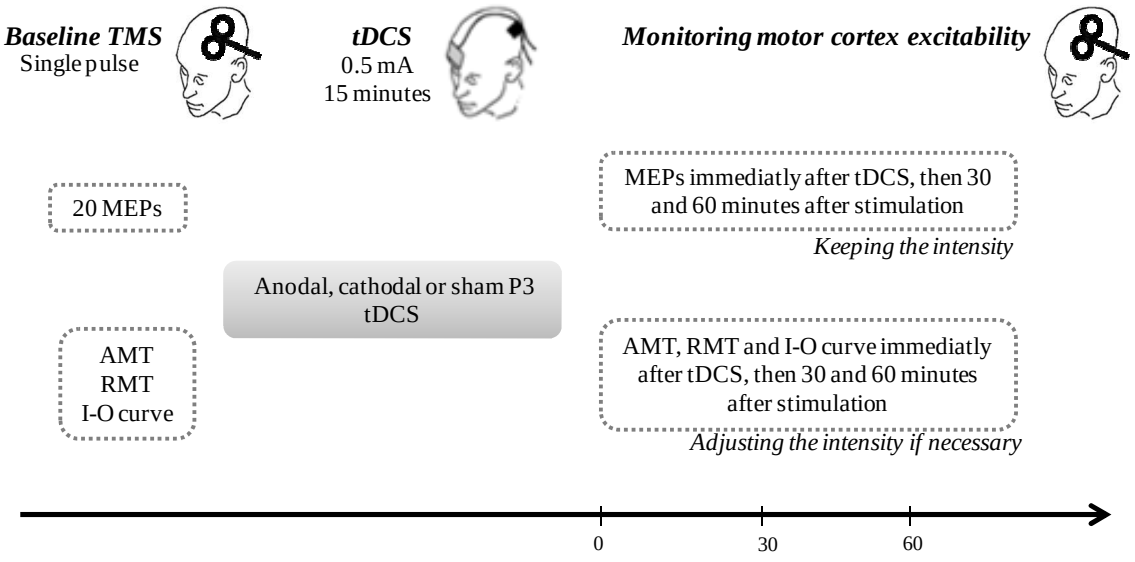


Figure 10. Course of the Experiment 1a. Motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) over the motor cortex representation of the right first dorsal interosseus muscle (FDI) were recorded at 1 mV intensity. Active motor threshold (AMT) and resting motor threshold (RMT) were also determinated. Input – output curve (I-O curve) was recorded by 100%, 110%, 130% and 150% of resting motor threshold (baseline). Then, transcranial direct current stimulation (tDCS) (anodal, cathodal or sham) was administered over P3. Immediately after the stimulation, 20 MEPs, AMT, RMT and I-O curve were measured, then thirty and sixty minutes later. TMS protocols obtained for baseline measures were used for monitoring.

3.4.2 Experiment 1b

Spatial specificity of the effects of parietal transcranial direct current stimulation on primary motor cortex excitability

Thirteen right-handed healthy subjects, 7 men and 6 women, aged 24 to 54 years (mean age = 28.6 ± 8.0 years), participated in this study. The general procedure was identical to that of experiment 1a. All subjects received anodal, cathodal and sham transcranial direct current stimulation over P3, and anodal or cathodal trancranial direct current stimulation over 3 cm lateral or posterior to P3 after obtaining baseline motor evoked potentials, as outlined above. After transcranial direct current stimulation, motor evoked potentials elicited by single pulse of transcranial magnetic stimulation were registered every five minutes for 30 minutes (0, 5, 10, 15, 20, 25, 30 min), and then every 30 minutes (60, 90, 120 min) until 2 hours (Figure 11).

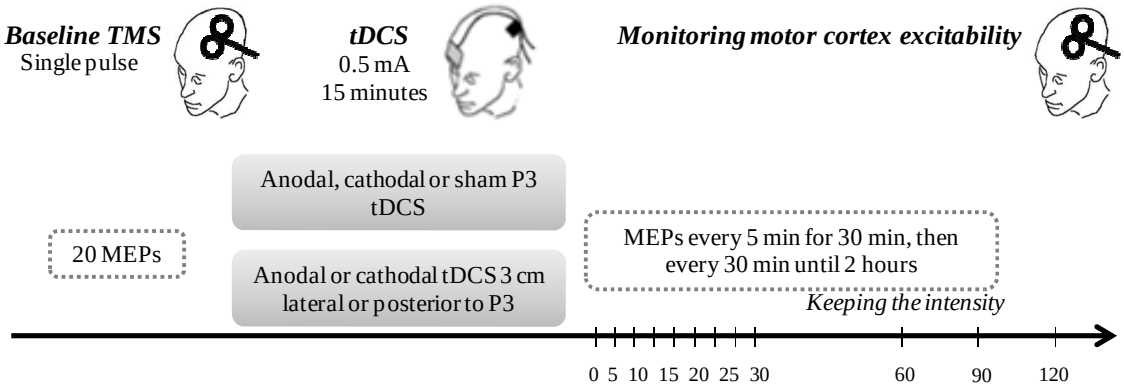


Figure 11. Course of the Experiment 1b. Motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) over the motor cortex representation of the right first dorsal interosseus muscle (FDI) were recorded at 1 mV intensity (baseline). Then, transcranial direct current stimulation (tDCS) (anodal, cathodal or sham) was administered over P3, and 3 cm lateral and 3 cm posterior to P3. Immediately after the stimulation, 20 MEPs were recorded, and every five minutes for thirty minutes. The same protocol was repeated every thirty minutes during two hours. TMS with the parameters obtained for baseline measures was used for monitoring.

3.4.3 Experiment 2a

Short interval intracortical inhibition and intracortical facilitation changes induced by parietal transcranial direct current stimulation

Fifteen right-handed healthy subjects, 5 men and 10 women, aged 19 to 31 years (mean age= 25.7 ± 3.4 years), participated in this study. Two high-power Magstim 200 machines, which were connected via a Bistim-module, were used in this experiment. Primary motor cortex excitability was first monitored by determination of active motor threshold as described above, and 20 motor evoked potentials were registered to determinate transcranial magnetic stimulation intensity resulting in ~1mV peak-to-peak amplitude motor evoked potential of the right first dorsal interosseus muscle before transcranial direct current stimulation (Figure 12).

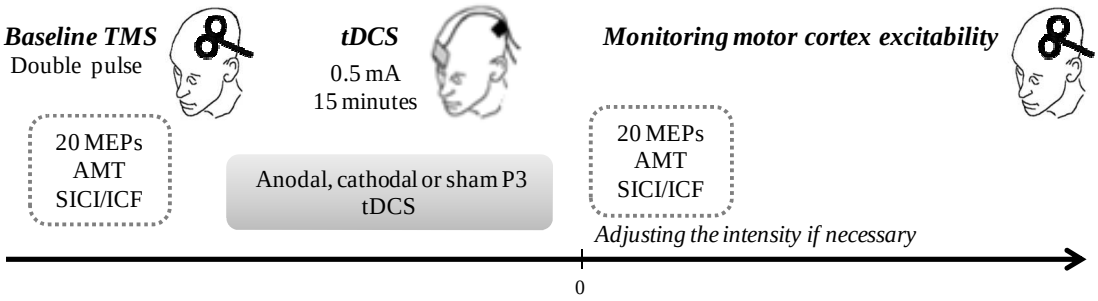


Figure 12. Course of the Experiment 2a. Motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) over the motor cortex representation of the right first dorsal interosseus muscle (FDI) were recorded at 1 mV intensity. Short-latency intracortical inhibition and intracortical facilitation (SICI/ICF) elicited by double-pulse transcranial magnetic stimulation over the motor cortex representation of the first dorsal interosseus muscle were recorded by a conditioning stimulus (CS) of 70% of the active motor threshold (AMT) and a test stimulus (TS) at 1 mV motor evoked potential amplitude size (baseline).Then, transcranial direct current stimulation (tDCS) (anodal, cathodal or sham) was administered over P3. Immediately after the stimulation, 20 MEPs at 1 mV intensity and SICI/ICF were recorded again. TMS with the parameters obtained for baseline measures was used for monitoring.

Short interval intracortical inhibition and intracortical facilitation were measured by the paired-pulse transcranial magnetic stimulation protocol described above. After that, anodal, cathodal and sham transcranial direct current stimulation were applied over P3 for 15 min in different sessions. Immediately after transcranial direct current stimulation, primary motor cortex excitability was monitored again by active motor threshold and motor evoked potentials of about 1 mV peak-to-peak amplitude, and, then, short interval intracortical inhibition and intracortical facilitation were recorded. Single pulse motor evoked potential and active motor threshold intensities were adjusted when necessary (Figure 12).

3.4.4 Experiment 2b

Parietal-motor cortical connectivity changes induced by parietal transcranial direct current stimulation

Fifteen right-handed healthy subjects participated in this study. As in the previous experiment, two high-power Magstim 200 machines connected via a Bistim-module were used. Primary motor cortex excitability was first monitored by single test-pulse transcranial magnetic stimulation obtained with a small eight-shaped coil (external diameter 50 mm). Twenty motor evoked potentials were registered to determinate transcranial magnetic stimulation intensity resulting in ~1mV peak-to-peak amplitude motor evoked potential of the right dorsal interosseus muscle before the stimulation. Then, resting motor threshold was determined as described above by single test-pulse transcranial magnetic stimulation using a small eight-shaped coil (external diameter 70 mm). The paired-pulse twin coil transcranial magnetic stimulation protocol used to

record short interval intracortical inhibition and intracortical facilitation was the same as described above, with the exception that the CS was delivered by the coil placed over P3, and the test pulse by the other coil positioned over the primary motor cortex. Then, anodal, cathodal and sham transcranial direct current stimulation of 0.5 mA was applied over P3 for 15 min in different sessions, as described above. Immediately after transcranial direct current stimulation termination, primary motor cortex excitability was monitored by determination of resting motor threshold and motor evoked potentials of about 1 mV peak-to-peak amplitude, and then short interval intracortical inhibition and intracortical facilitation were recorded. Single pulse motor evoked potential and resting motor threshold intensities were adjusted when necessary (Figure 13).

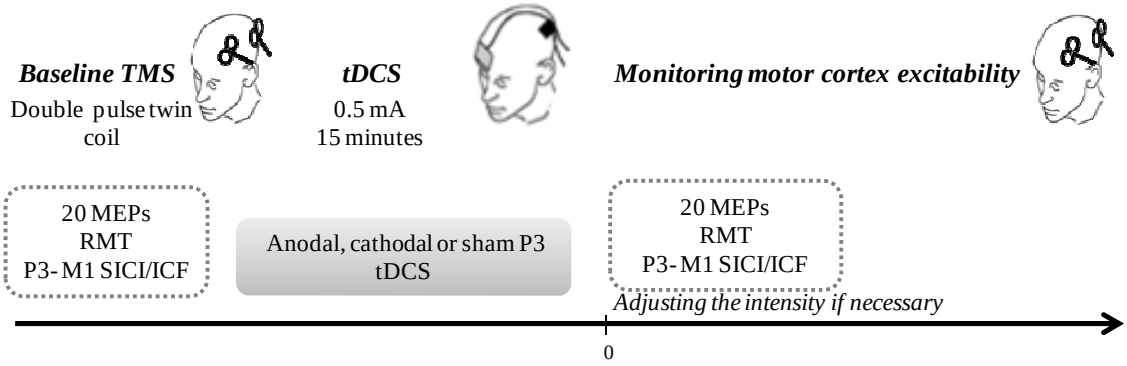


Figure 13. Course of the Experiment 2b. Motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS) over the motor cortex representation of the right first dorsal interosseus muscle (FDI) were recorded at 1 mV intensity. A twin-coil transcranial magnetic stimulation protocol was used to study P3-M1 connectivity by parieto-motor short latency intracortical inhibition and intracortical facilitation (pmSICI/pmICF). Test stimulus (TS) was applied over the motor cortex representation of the first dorsal interosseus muscle at 1 mV, and conditioning stimulation (CS) was applied over P3 at 90% of the resting motor threshold (RMT) (baseline), then transcranial direct current stimulation (tDCS) (anodal, cathodal or sham) was administered over P3. Immediately after the stimulation, 20 MEPs were recorded and pmSICI/pmICF were again recorded. TMS with the parameters obtained for baseline measures was used for monitoring.

3.5 DATA ANALYSIS

For the single pulse motor evoked potentials measures, individual means of peak-to-peak motor evoked potential amplitudes (mV) were calculated off-line, and baseline-standardized individual peak-to-peak motor evoked potential amplitudes were calculated for each stimulation condition and for each time-bin covering the recordings before and after transcranial direct current stimulation. For short interval intracortical inhibition and intracortical facilitation protocols, mean motor evoked potential amplitudes were standardized to the respective single-pulse condition. Individual means were calculated for each interstimulus stimulation and stimulation condition.

In all experiments the Mauchley-test for proving sphericity was applied. The Greenhouse-Geisser correction was applied to correct for non-sphericity, if necessary. Baseline differences between anodal, cathodal, and sham transcranial direct current stimulation were explored for non-standardized motor evoked potential amplitudes via a one-factorial analysis of the variance (ANOVA) with the factor “tDCS condition”. A repeated measure ANOVA with the dependent variable percentage of maximal stimulator output was conducted for motor thresholds. Critical level of significance was set to $p < 0.05$ for all tests. Post hoc tests were not corrected for multiple comparisons. All analyses were carried out using SPSS software.

In experiment 1a, for the respective repeated measure ANOVA, baseline standardized motor evoked potential amplitudes from each subject served as the dependent variable, and transcranial direct current stimulation condition (anodal/cathodal) and time as within subject factors. For input-output curve, transcranial magnetic stimulation intensity served as an additional factor. In case of significant results, post hoc two-tailed paired samples *Student´s* t-tests were conducted to compare

motor evoked potential amplitude alterations of the respective time bins versus baseline, or within a time bin between transcranial direct current stimulation conditions. A one-way ANOVA, with time as a repeated measure factor and motor evoked potential as dependent variable, was conducted for sham stimulation in a subgroup of subjects. A two-way repeated measure ANOVA was conducted for input-output curve, with time and transcranial magnetic stimulation intensity as repeated measure factor.

For the repeated measure ANOVA of experiment 1b, baseline standardized motor evoked potential amplitudes from each subject served as the dependent variable, and transcranial direct current stimulation conditions condition (anodal/cathodal/sham), time and electrode position served as within subject factors. In case of significant results of the ANOVA, post hoc two-tailed paired samples *Student´s* t-tests were conducted to compare motor evoked potential amplitude alterations of the respective time bins versus baseline, or within a time bin between transcranial direct current stimulation conditions.

Motor evoked potential amplitudes recorded from 1a and 1b experiments were also calculated together for anodal and cathodal stimulation and for each time bin at 0 min, 30 min and 60 min, and data were analyzed by a two-way ANOVA. Baseline standardized motor evoked potential amplitudes from each subject served as dependent variable. Transcranial direct current stimulation conditions (anodal and cathodal) and time served as within subject factors. Post hoc two-tailed paired samples *Student´s* t-tests were conducted to compare motor evoked potential amplitude alterations of the respective time bins versus baseline.

Two separate ANOVAs were conducted for experiments 2a and 2b. For the respective repeated measure ANOVAs, single transcranial magnetic stimulation test pulse-standardized double-pulse transcranial magnetic stimulation-elicited motor

evoked potential amplitudes from each subject served as the dependent variable, and transcranial direct current stimulation condition (anodal/cathodal/sham), time (before and after transcranial direct current stimulation) and interstimulus intervals (pooled data for 2-4, 6-8 and 10-15 ms) served as repeated measure factors. When the results were significant, post hoc two-tailed paired samples *Student´s* t-tests were conducted to compare motor evoked potential amplitude alterations of the respective interstimulus intervals versus baseline or sham, or within an interstimulus interval between transcranial direct current stimulation conditions.

4. RESULTS

None of the subjects reported any relevant adverse effects during or after the study in any of the experiments. Baseline values of motor evoked potentials, resting motor threshold, active motor threshold, and short-latency intracortical inhibition and intracortical facilitation did not differ between sessions in the respective experiments.

4.1 EXPERIMENT 1a

The percentage of maximum stimulator output for motor thresholds and the absolute values for motor evoked potential baselines for all experimental sessions are shown in Table 1. The results of the ANOVA for baselines and motor thresholds did not differ between experimental sessions. Resting motor threshold and active motor threshold did not differ after transcranial direct current stimulation conditions (Table 2).

Table 1. Baselines and motor thresholds values before and after transcranial direct current stimulation (tDCS) over P3 of the experiment 1a. Data are presented as mean ± standard deviation (SD). Abbreviations: AMT= active motor threshold; F= female; M= male; n= number of participants; RMT= resting motor threshold; SI 1mv= transcranial magnetic stimulation intensity adjusted to elicit ~ 1 mV peak to peak amplitude of motor evoked potentials (MEPs); *= Percentage of maximum stimulator output (%MSO).

						Before tDCS	
Experimental session	**n**	**Sex (M/F)**	**Age**	**SI 1mv (%)***	**Baseline MEP amplitude (mV)**	**RMT (%)***	**AMT (%)***
Anodal tDCS over P3	14	7F/7M	28.3 ± 9.4	45.7 ± 6.2	1.03 ± 0.09	39.9 ± 4.7	31.4 ± 5.2
Cathodal tDCS over P3	14	7F/7M	28.3 ± 9.4	46 ± 6.5	1.06 ± 0.07	40.1 ± 6.6	32.5 ± 6.3
Sham tDCS over P3	6	3F/3M	28.3 ± 9.4	47.1 ± 3.7	1.09 ± 0.07	41.8 ± 3.8	32.1 ± 5.4
					After tDCS 0 min		
Anodal tDCS over P3						39.8±5.3	31.9±5.4
Cathodal tDCS over P3						40.4±5.7	32.5±5.2
Sham tDCS over P3						41.6±3.6	32.5±4.8
					After tDCS 30 min		
Anodal tDCS over P3						40.6±5.5	31.5±5.9
Cathodal tDCS over P3						40.5±6.4	32.3±5.5
Sham tDCS over P3						41.3±3.6	32.6±5.2
					After tDCS 60 min		
Anodal tDCS over P3						40.4±5.1	31.5±6.3
Cathodal tDCS over P3						40.6±6.0	32.5±5.8
Sham tDCS over P3						41.1±4.8	33±4.0

Table 2. Results of the analysis of variance (ANOVA) tests of the experiment 1a. One-way ANOVAs were calculated for baselines values and motor thresholds, active motor threshold (AMT) sham and resting motor threshold (RMT) sham. Two-way ANOVAs repeated measures were calculated for AMT, RMT and motor evoked potentials (MEPs) before and after stimulation. Three-way repeated measures ANOVAs were calculated for input-output curve (I-O Curve). Abbreviations: d.f.= degrees of freedom; tDCS= transcranial direct current stimulation; TMS= transcranial magnetic stimulation. *= $P<0.05$.

Measurement	Factor	d.f	F-value	P-value
Baseline MEP amplitude (mV)				
	Experimental session	1	2.896	0.113
Motor threshold (MT)				
	MT Experimental sessions Anodal/Cathodal	1	0.179	0.679
	MT Experimental session sham	3	0.7	0.431
	RMT stimulation	1	0.538	0.476
	RMT time	3	0.776	0.514
	RMT stimulation x time	3	0.073	0.974
	RMT sham	3	0.484	0.518
	AMT stimulation	1	2.246	0.158
	AMT time	3	0.283	0.837
	AMT stimulation x time	3	0.424	0.737
	AMT sham	3	1.373	0.294
MEP				
	tDCS stimulation	1	14.3	0.002*
	TMS time	3	1.3	0.281
	tDCS stimulation x TMS time	3	5.9	0.002*
	Single test pulse TMS time (sham)	3	0.7	0.431
I-O Curve				
	tDCS stimulation	1	1.2	0.296
	TMS time	3	0.2	0.905
	TMS intensity	3	27.5	< 0.001*
	tDCS stimulation x TMS time	3	0.5	0.687
	Time x TMS intensity	9	1	0.471
	tDCS stimulation x TMS intensity	3	1.5	0.224
	TMS time x tDCS stimulation x TMS intensity	9	0.6	0.758
	TMS time (Sham)	3	1.5	0.265
	TMS intensity	3	32.1	< 0.001*
	TMS time x TMS intensity	1	9	0.474

The repeated-measures ANOVA conducted for single-test pulse motor evoked potential (Table 2) showed a significant main effect of polarity (P = 0.002) and a significant effect of the interaction between time and polarity (P = 0.002). The post-hoc tests show significant motor evoked potential enhancements versus baseline in the anodal condition immediately after transcranial direct current stimulation and 60 min

after stimulation ($P < 0.05$). In the cathodal transcranial direct current stimulation condition, motor evoked potential size was significantly reduced versus baseline only immediately after transcranial direct current stimulation ($P < 0.05$). The comparison between anodal versus cathodal transcranial direct current stimulation showed significant differences immediately after transcranial direct current stimulation, 30 and 60 min after stimulation ($P < 0.05$). The exact values of the post-hoc test for significant results are showed in the Table 3.

Table 3. Significant values for post hoc analysis of the experiment 1a. Abbreviation: tDCS= transcranial direct current stimulation.

Anodal tDCS vs Baseline	**Significance**	**Cathodal tDCS vs Baseline**	**Significance**	**Anodal vs Cathodal tDCS**	**Significance**
0 minutes	$P = 0.004$	0 minutes	$P = 0.004$	0 minutes	$P = 0.001$
60 minutes	$P = 0.002$	-	-	30 minutes	$P = 0.033$
-	-	-	-	60 minutes	$P = 0.007$

The one-way ANOVA conducted for sham transcranial direct current stimulation showed no significant effect for time ($P = 0.431$). Figure 14 shows the motor evoked potential alterations induced by anodal, cathodal and sham parietal transcranial direct current stimulation. For the input-output curve, the main effect of the stimulation intensity was significant in the three experimental sessions, but there was no significant effect due to transcranial direct current stimulation (Figure 15).

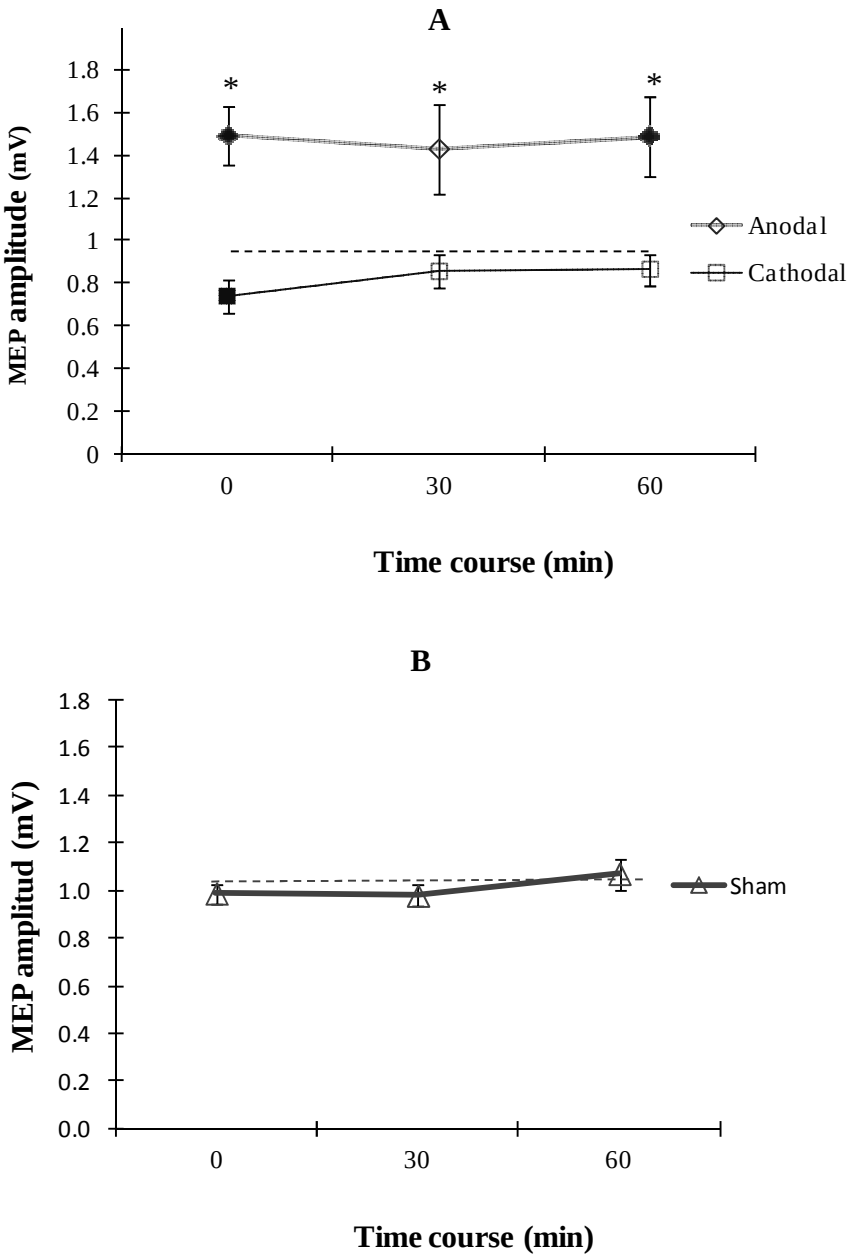


Figure 14. Time course (in minutes) of the motor evoked potential (MEP) amplitudes for anodal and cathodal transcranial direct current stimulation (tDCS) (A) and sham stimulation (B) over P3 (experiment 1a). (**A**) Compared to baseline values, MEPs were significantly larger in the anodal condition 0 min and 60 min (♦P < 0.05) after posterior parietal tDCS (P3), and significantly diminished in the cathodal condition 0 min after P3 tDCS (■*P* < 0.05). MEPs were significantly larger after anodal, compared to cathodal P3 tDCS, immediately, 30 min and 60 min (**P* < 0.05) after tDCS. Sham tDCS (**B**) did not result in any MEPs alterations. The dotted line indicates baseline MEP amplitude. (◊) Anodal tDCS. (□) Cathodal tDCS. (Δ) Sham tDCS. (♦) Anodal tDCS versus baseline significance. (■) Cathodal tDCS versus baseline significance. (*) Anodal versus cathodal tDCS significance. Error bars represent standard error of means (SEM).

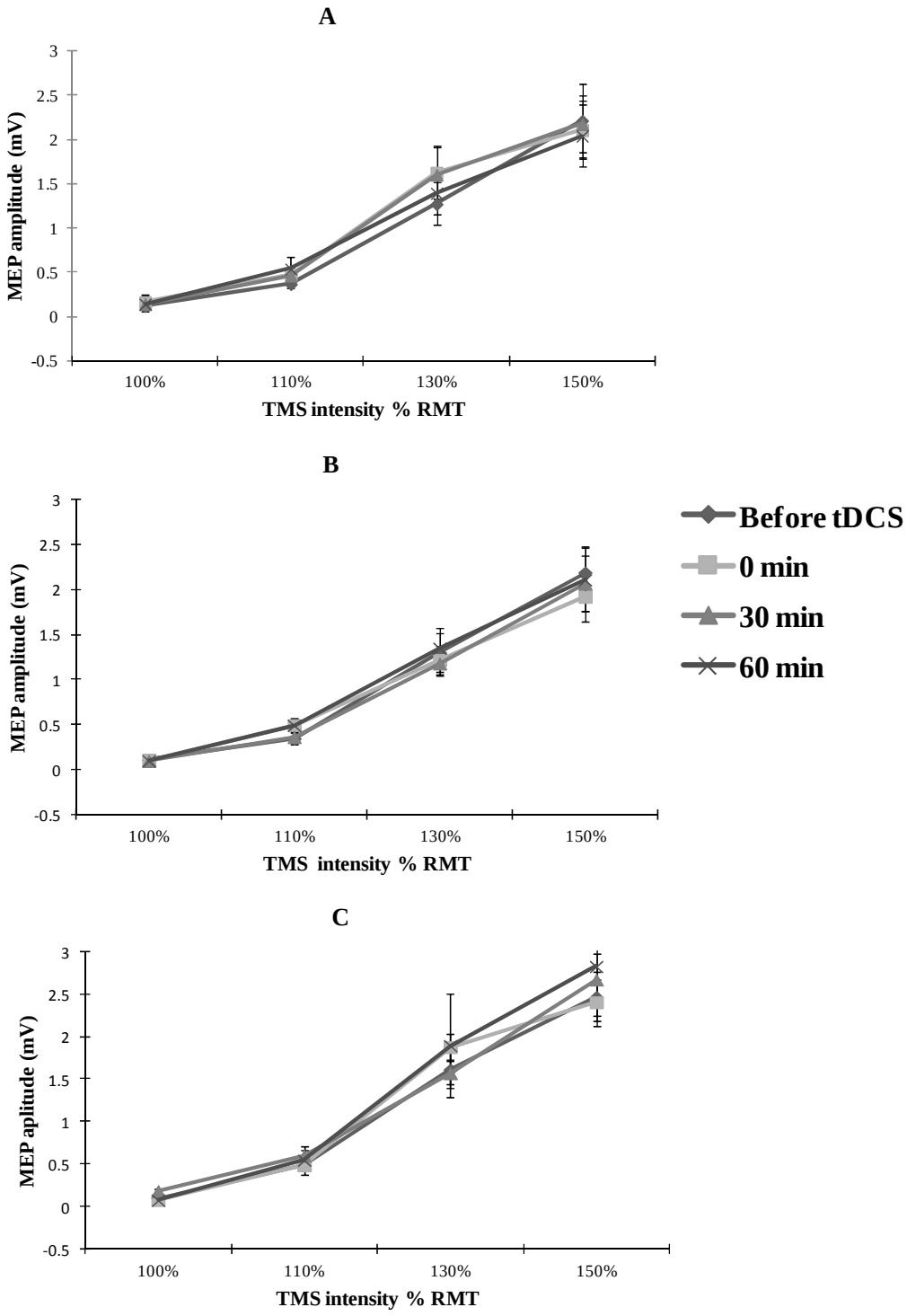


Figure 15. Input-output curve (I-O curve) for anodal (A), cathodal (B) and sham (C) transcranial direct current stimulation (tDCS) over P3 (experiment 1a). The I-O curve was recorded with TMS (♦) before tDCS and three times after stimulation at 0 min(■), 30 minutes (▲) and 60 minutes (×). The TMS intensity was adjusted at 100%, 110%, 130% and 150% of the resting motor threshold (RMT). No significant changes were observed after tDCS. Error bars represent standard error of means (SEM).

4.2 EXPERIMENT 1b

The percentage of maximum stimulator output for motor thresholds and the absolute values for motor evoked potential baselines for all experimental sessions are shown in Table 4. The results of the ANOVA for baselines and motor threshold did not differ between experimental sessions (Table 5).

Table 4. Baseline and motor thresholds values before transcranial direct current stimulation (tDCS) of the experiment 1b. Data are presented as mean ± standard deviation (SD). Abbreviation: F= female; M= male; n= number of participants; SI1mv= TMS intensity adjusted to elicit ~ 1 mV peak to peak amplitude of motor evoked potentials (MEPs). *= Percentage of maximum stimulator output (%MSO).

Experimental session	n	Sex (M/F)	Age	SI 1mv (%)*	Baseline MEP amplitude (mV)
Anodal tDCS over P3	13	6F/7M	28.6 ± 8.0	50.2 ± 9.9	1.05 ± 0.02
Cathodal tDCS over P3	13	6F/7M	28.6 ± 8.0	47.9 ± 10.2	1.03 ± 0.06
Sham tDCS over P3	13	6F/7M	28.6 ± 8.0	50.0 ± 10.7	1.06 ± 0.10
Anodal tDCS over 3 cm post toP3	13	6F/7M	28.6 ± 8.0	51.3 ± 11.3	1.02 ± 0.07
Cathodal tDCS over 3 cm post toP3	13	6F/7M	28.6 ± 8.0	49.8 ± 10.5	1.02 ± 0.10
Anodal tDCS over 3 cm lat toP3	13	6F/7M	28.6 ± 8.0	49.3 ± 10.2	1.01 ± 0.06
Cathodal tDCS over 3 cm lat toP3	13	6F/7M	28.6 ± 8.0	49.6 ± 10.1	0.99 ± 0.07

The repeated-measures ANOVA conducted for single-test pulse motor evoked potential showed a significant main effect of transcranial direct current stimulation (P = 0.015) and transcranial magnetic stimulation time (P = 0.048), and a significant interaction between electrode transcranial direct current stimulation position and transcranial direct current stimulation itself (P < 0.001), electrode transcranial direct current stimulation position and transcranial magnetic stimulation time (P = 0.020), and electrode transcranial direct current stimulation position, transcranial direct current stimulation itself and time (P = 0.006) (Table 5).

Table 5. Results of the analysis of variance (ANOVA) tests from experiment 1b. One-way ANOVAs were calculated for baselines values and motor thresholds. A three-way repeated measures ANOVA was calculated for motor evoked potentials (MEPs) before and after stimulation. For the MEPs recorded before and after stimulation of the experiments 1a and 1b together, a two-way repeated measures ANOVA was calculated. Abbreviations: d.f.= degrees of freedom; tDCS: transcranial direct current stimulation; TMS= transcranial magnetic stimulation. *= P<0.05.

Measurement	Factor	d.f	F-value	P-value
Baseline MEP amplitude (mV)				
	Experimental session	6	1.123	0.357
Motor threshold (MT)				
	MT Experimental sessions	6	1.196	0.318
MEP				
	tDCS position	2	1.695	0.205
	tDCS stimulation	2	5.019	0.015*
	TMS time	10	1.928	0.048*
	tDCS position x tDCS stimulation	4	7.822	<0.001*
	tDCS position x TMS time	20	1.814	0.020*
	tDCS stimulation x TMS time	20	0.967	0.503
	tDCS position x tDCS stimulation x TMS time	40	1.698	0.006*
Results of the ANOVA. MEPs experiments 1a and 1b				
Measurement	**Factor**	**d.f**	**F-value**	**P-value**
MEP				
	tDCS stimulation	1	37.927	<0.001*
	TMS time	1	6.043	0.021*
	TMS time x tDCS stimulation	1	22.025	<0.001*

Post-hoc tests revealed motor evoked potential enhancements in the anodal condition versus baseline immediately after transcranial direct current stimulation over P3 (0 min), as well as 5, 15, 20, 30, 60, 90, and 120 min after stimulation ($P < 0.05$). In the cathodal transcranial direct current stimulation over P3 condition, motor evoked potential size was significantly reduced versus baseline at 5, 10, 15, 20, 25, 30, 60, 90, and 120 min after stimulation ($P < 0.05$). The post-hoc tests showed a significant motor evoked potential increase in the anodal transcranial direct current stimulation over P3 condition compared with sham at 30, 60, 90, and 120 min after transcranial direct current stimulation ($P < 0.05$). Regarding the cathodal transcranial direct current stimulation over P3 condition compared with sham there was a significant motor evoked potential decrease at 5, 10, 15, 20, 25, 30, 60 and 120 min after transcranial

direct current stimulation ($P < 0.05$). Table 6 shows the significant exact values for the post-hoc test results.

Table 6. Significant values for post hoc analysis of the experiment 1b. Abbreviations: tDCS over P3= transcranial direct current stimulation over the posterior parietal cortex region of the international 10-20 electroencephalography system.

Anodal tDCS over P3 vs Baseline	**Significance**	**Cathodal tDCS over P3 vs Baseline**	**Significance**
0 minutes	$P = 0.036$	5 minutes	$P = 0.030$
5 minutes	$P = 0.025$	10 minutes	$P < 0.001$
15 minutes	$P = 0.023$	15 minutes	$P = 0.011$
20 minutes	$P = 0.007$	20 minutes	$P = 0.009$
30 minutes	$P = 0.002$	25 minutes	$P = 0.038$
60 minutes	$P = 0.019$	30 minutes	$P = 0.008$
90 minutes	$P = 0.003$	60 minutes	$P = 0.034$
120 minutes	$P = 0.005$	90 minutes	$P = 0.043$
-	-	120 minutes	$P = 0.021$
Anodal tDCS over P3 vs sham	**Significance**	**Cathodal tDCS over P3 vs sham**	**Significance**
30 minutes	$P = 0.004$	5 minutes	$P = 0.012$
60 minutes	$P = 0.025$	10 minutes	$P = 0.001$
90 minutes	$P = 0.002$	15 minutes	$P = 0.008$
120 minutes	$P = 0.002$	20 minutes	$P = 0.002$
-	-	25 minutes	$P = 0.009$
-	-	30 minutes	$P = 0.001$
-	-	60 minutes	$P = 0.037$
-	-	120 minutes	$P = 0.004$
Anodal tDCS over 3 cm posterior to P3 vs baseline	**Significance**	**Anodal tDCS over 3 cm posterior to P3 vs sham**	**Significance**
90 minutes	$P = 0.034$	10 minutes	$P = 0.025$
120 minutes	$P = 0.004$	-	-
Significative values for the post-hoc test. Experiment 1a and 1b			
Anodal tDCS over P3 vs Baseline	**Significance**	**Cathodal tDCS over P3 vs Baseline**	**Significance**
0 minutes	$P < 0.001$	0 minutes	$P = 0.019$
30 minutes	$P < 0.001$	30 minutes	$P = 0.012$
60 minutes	$P < 0.001$	60 minutes	$P = 0.003$

Figure 16 shows the motor cortex excitability changes after anodal, cathodal and sham anodal transcranial direct current stimulation over the posterior parietal cortex region (P3) of the international 10-20 electroencephalography system.

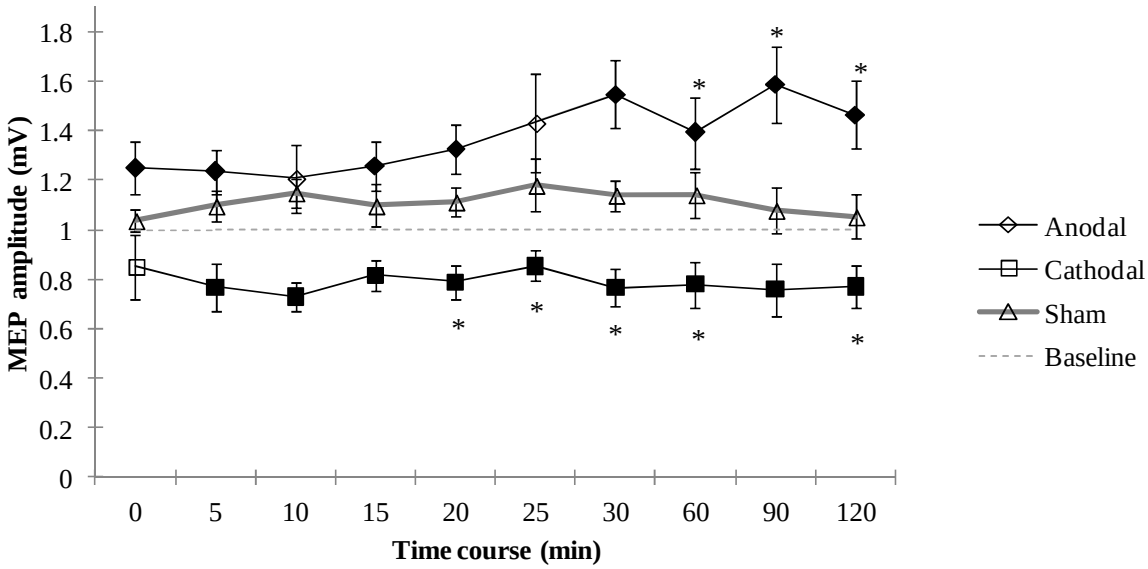


Figure 16. Time course (in minutes) of the motor evoked potential (MEP) amplitudes for anodal, cathodal and sham transcranial direct current stimulation (tDCS) over the posterior parietal cortex (P3) (experiment 1b). tDCS over P3 resulted in an MEP amplitude enhancement for anodal (♦P < 0.05), and MEP decrease for cathodal tDCS (■P < 0.05), compared to baseline. The dotted line indicates baseline MEP amplitude. (◊) Anodal tDCS. (∈) Cathodal tDCS. (Δ) Sham tDCS. (♦) Anodal tDCS versus baseline significance. (■) Cathodal tDCS versus baseline significance. (*) Anodal or cathodal tDCS versus sham significance. Error bars represent standard error of means (SEM).

Post-hoc tests for the 3 cm posterior to P3 position (Figure 17) showed motor evoked potential enhancements in the anodal condition compared to baseline at 90 and 120 min ($P < 0.05$), and sham at 10 min ($P < 0.05$). Cathodal condition over 3 cm posterior to P3 did not result in any significant change. The results of post-hoc tests for

the 3 cm lateral to P3 position indicated no significant motor evoked potential changes in anodal or cathodal transcranial direct current stimulation conditions (Figure 18). In the sham condition no motor evoked potential changes were found.

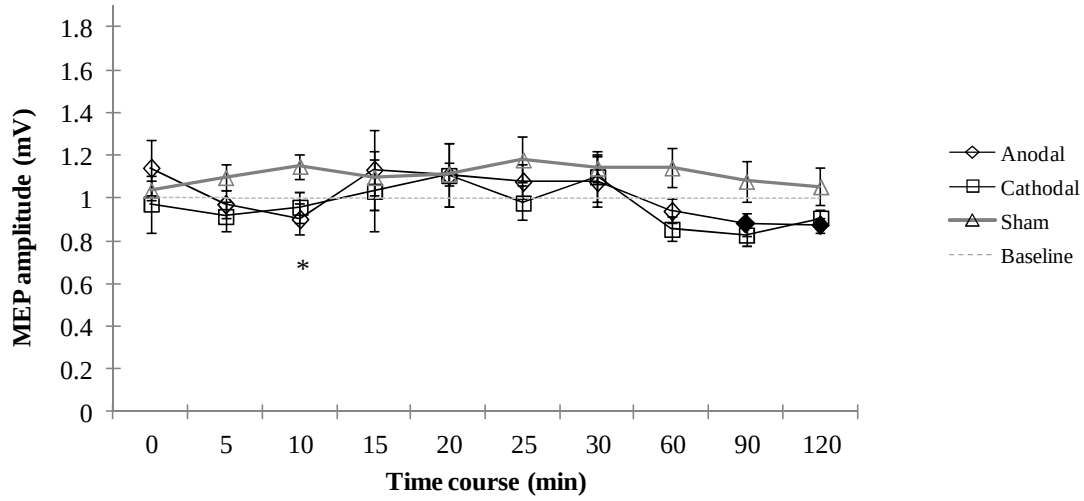


Figure 17. Time course (in minutes) of the motor evoked potential (MEP) amplitudes for anodal, cathodal and sham transcranial direct current stimulation (tDCS) over 3 cm posterior to the posterior parietal cortex (P3) (experiment 1b). Compared to baseline, MEPs were significantly diminished after anodal (♦ $P < 0.05$) and cathodal tDCS (■$P < 0.05$), and compared to sham, MEPs were significantly decreased in the anodal (*$P < 0.05$) and cathodal (*$P < 0.05$) conditions. The dotted line indicates baseline MEP amplitude. (◊) Anodal tDCS. (∈) Cathodal tDCS. (Δ) Sham tDCS. (♦) Anodal tDCS versus baseline significance. (■) Cathodal tDCS versus baseline significance. (*) Anodal or cathodal tDCS versus sham significance. Error bars represent standard error of means (SEM).

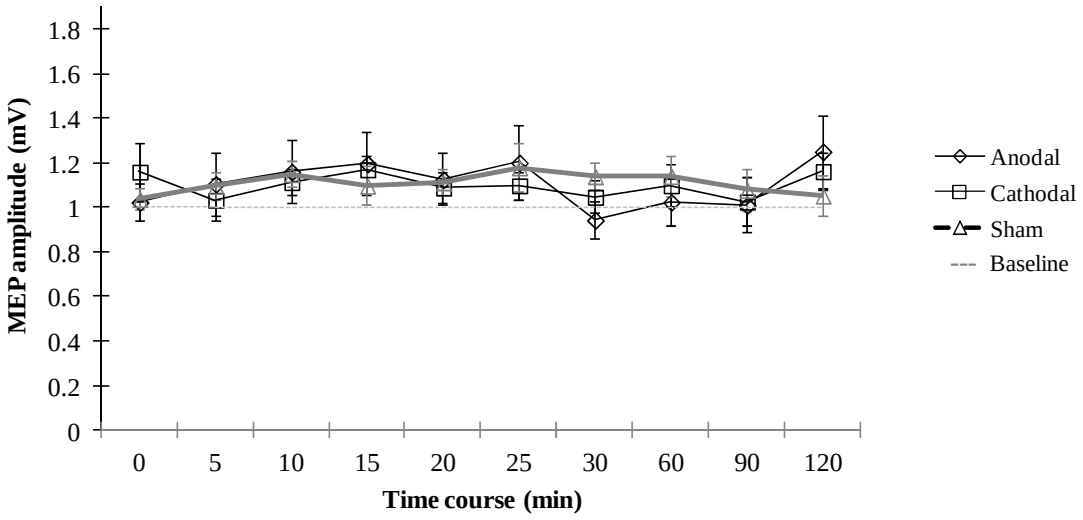


Figure 18. Time course (in minutes) of the motor evoked potential (MEP) amplitudes for anodal, cathodal and sham transcranial direct current stimulation (tDCS) over 3 cm lateral to the posterior parietal cortex (P3) (experiment 1b). tDCS applied 3 cm lateral to P3 did not result in any MEPs alterations. The dotted line indicates baseline MEP amplitude. (◊) Anodal tDCS. (∈) Cathodal tDCS. (Δ) Sham tDCS. Error bars represent standard error of means (SEM).

The two-way ANOVA for the motor evoked potentials of the experiments 1a and 1b showed a significant main effect of transcranial direct current stimulation (P = 0.001) and transcranial magnetic stimulation time (P = 0.021), and a significant effect of the interaction between transcranial magnetic stimulation time and transcranial direct current stimulation ($P < 0.001$) (Table 5). The post-hoc tests showed significant motor evoked potential enhancements versus baseline in the anodal condition at 0, 30 and 60 min after stimulation ($P < 0.05$). In the cathodal transcranial direct current stimulation condition, motor evoked potential size was significantly reduced versus baseline at 0, 30 and 60 min after this stimulation ($P < 0.05$) (Figure 19). Table 6 shows the significant exact values for the post-hoc test results.

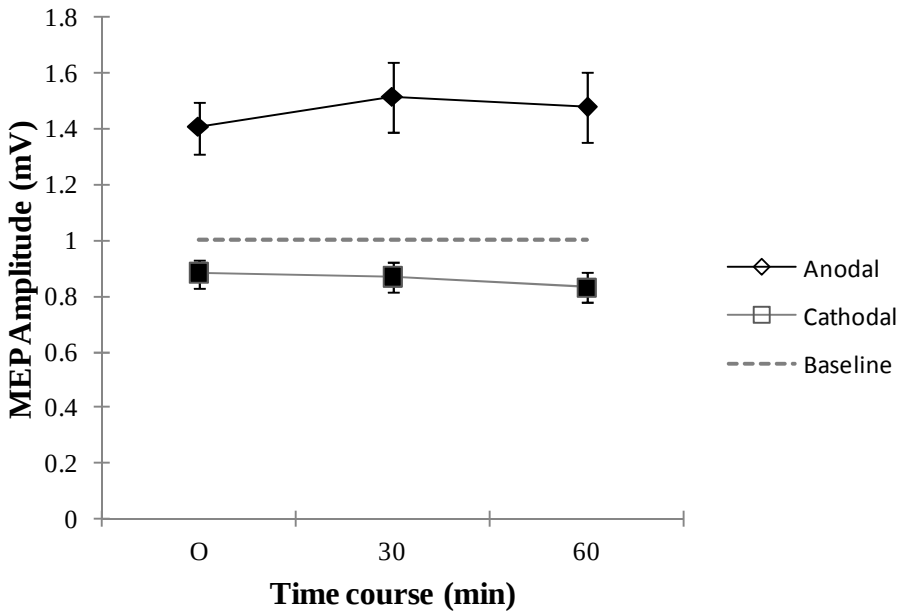


Figure 19. Time course (in minutes) of the motor evoked potential (MEP) amplitudes for anodal and cathodal transcranial direct current stimulation (tDCS) over posterior parietal cortex (P3) (experiments 1a and 1b taken together). Compared to baseline values, MEPs amplitudes were significantly larger in the anodal condition at 0 min, 30 min and 60 min (♦$P < 0.05$) after P3 tDCS, and significantly diminished in the cathodal condition at 0 min, 30 min and 60 min after P3 tDCS (■$P < 0.05$). The dotted line indicates baseline motor evoked potential amplitude. (◊) Anodal tDCS. (□) Cathodal tDCS. (♦) Anodal tDCS versus baseline significance. (■) Cathodal tDCS versus baseline significance. Error bars represent standard error of means (SEM).

4.3 EXPERIMENT 2a

The percentage of maximum stimulator output for motor thresholds and the absolute values for motor evoked potential baselines for all the experimental sessions are shown in Table 7. The results of the ANOVA for baselines and motor threshold did not differ between experimental sessions (Table 8).

Table 7. Baseline and motor thresholds values before and after transcranial direct current stimulation (tDCS) of the experiment 2a. Data are presented as mean ± standard deviation (SD). Abbreviation: AMT= Active motor threshold; F= female; M= male; n= number of participants; P3= posterior parietal cortex region of the international 10-20 electroencephalography system; SI 1mv= transcranial magnetic stimulation intensity adjusted to elicit ~ 1 mV peak to peak amplitude of motor evoked potentials (MEPs). *= Percentage of maximum stimulator output (%MSO).

				Before tDCS		
Experimental session	**n**	**Sex (M/F)**	**Age**	**SI $_{1mv}$ (%)***	**AMT (%)***	**Baseline MEP amplitude (mV)**
Anodal tDCS over P3	15	10F/5M	25.7 ± 3.4	42.0 ± 5.6	29.6 ± 4.2	1.18±0.15
Cathodal tDCS over P3	15	10F/5M	25.7 ± 3.4	41.3 ± 5.5	30.3 ± 2.9	1.16±0.13
Sham tDCS over P3	15	10F/5M	25.7 ± 3.4	42.6 ± 5.4	29.6 ± 4.7	1.12±0.17
				After tDCS		
Anodal tDCS over P3				41.4 ± 6.5	29.8 ± 3.8	1.12±0.16
Cathodal tDCS over P3				40.8 ± 5.6	29.6 ± 4.0	1.12±0.18
Sham tDCS over P3				42.2 ± 5.7	29.8 ± 4.2	1.14±0.14

The repeated-measures ANOVA (Table 8) shows a significant main effect of the interstimulus interval ($P < 0.001$) and a significant transcranial direct current stimulation condition and time interaction ($P = 0.007$). The results of the post-hoc tests revealed significant motor evoked potential enhancements in the anodal condition versus baseline and sham stimulation in the interstimulus intervals 5-7 and 10-15 ms ($P < 0.05$) Table 9 shows the significant exact values for the post-hoc test results. Figure 20 shows the short latency intracortical inhibition and intracortical facilitation results in the anodal condition. Post-hoc tests for cathodal transcranial direct current stimulation indicated no significant differences versus baseline or sham (Figure 21). In the sham condition no motor evoked potential changes were found (Figure 22).

Table 8. Results of the analysis of variance (ANOVA) tests of the experiment 2a. A two-way repeated-measures ANOVAs were calculated for baselines values and motor thresholds. A three-way repeated-measures ANOVAs were calculated for motor evoked potentials (MEP). Abbreviations: AMT = active motor threshold; d.f.= degrees of freedom; ISIs= interstimulus intervals; SICI/ICF= short latency intracortical inhibition and intracortical facilitation; tDCS= transcranial direct current stimulation; TMS= transcranial magnetic stimulation. *P<0.05.

Measurement	Factor	d.f	F-value	P-value
Baseline MEP amplitude (mV)				
	Experimental session	2	0.091	0.913
	Time	1	0.983	0.338
	Experimental session x time	2	0.704	0.503
Motor threshold (MT)				
	MT Stimulation	2	0.939	0.403
	MT Time	1	1.402	0.256
	MT Stimulation x time	2	0.02	0.98
	AMT Stimulation	2	0.061	0.941
	AMT Time	1	0.327	0.576
	AMT Stimulation x time	2	0.693	0.509
SICI/ICF				
	tDCS stimulation	2	0.187	0.83
	TMS time	1	1.839	0.197
	ISIs	2	33.71	<0.001*
	tDCS stimulation x TMS time	2	5.929	0.007*
	tDCS stimulation x ISIs	4	0.959	0.437
	Time x ISIs	2	1.773	0.188
	Stimulation x TMS time x ISIs	4	0.042	0.213

Table 9. Significant values for post hoc test of the experiment 2a. ISIs= interstimulus intervals (expressed in ms); tDCS over P3= transcranial direct current stimulation over the posterior parietal cortex region of the international 10-20 electroencephalography system.

Anodal tDCS over P3 vs Baseline	Significance	Anodal tDCS over P3 vs Sham	Significance
ISIs 5 -7	P = 0.014	ISIs 5 -7	P = 0.034
ISIs 10 - 15	P = 0.005	ISIs 10 - 15	P = 0.039

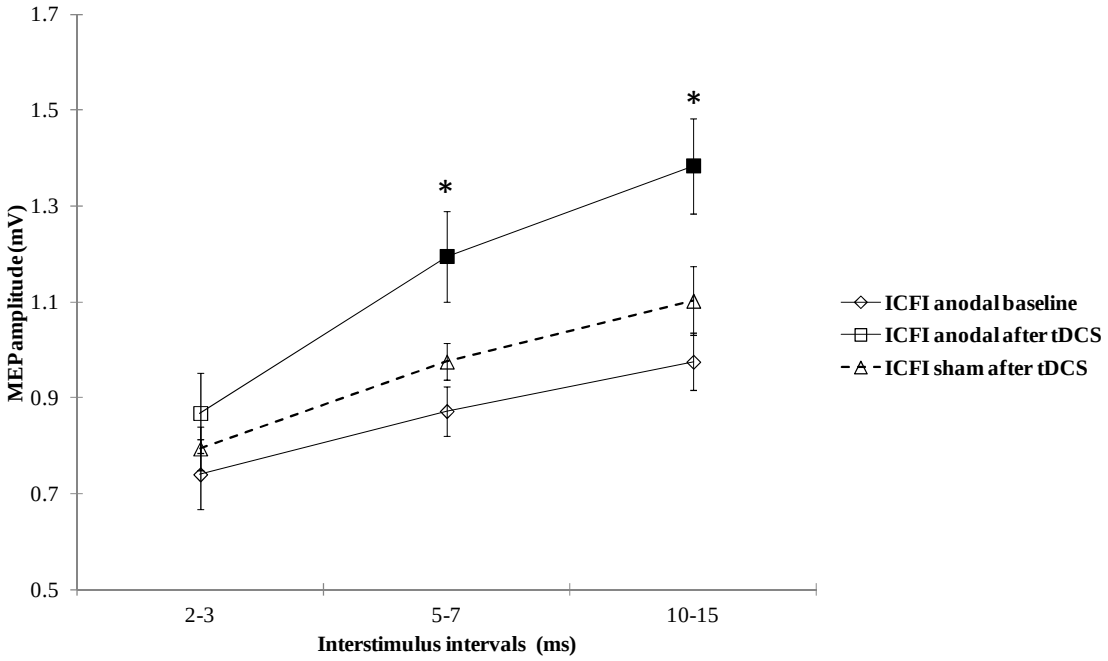


Figure 20. Motor cortex short latency intracortical inhibition/intracortical facilitation (SICI/ICF) for anodal and sham transcranial direct current stimulation (tDCS) (experiment 2a). The conditioning stimulus (CS) was set to an intensity of 70 % of the active motor threshold (AMT). Test stimulus (TS) intensity was adjusted to evoke a MEP of about 1 mV peak-to-peak amplitude. TS amplitude was adjusted after tDCS when necessary. Interstimulus intervals (ISIs) were 2, 3, 5, 7, 10 and 15 ms (arranged in the abscissa in three groups of intervals of 2-3, 5-7 and 10-15 ms). Significant ICF alterations were present in the anodal condition for the interstimulus intervals of 5-7 and 10-15 ms, compared to baseline (■$P < 0.05$) and sham (*$P < 0.05$). (◊) Before tDCS condition. ( ) After tDCS condition. (Δ) After sham tDCS. (■) Anodal tDCS vs. baseline significance. (*) Anodal tDCS versus sham significance. Error bars represent standard error of means (SEM).

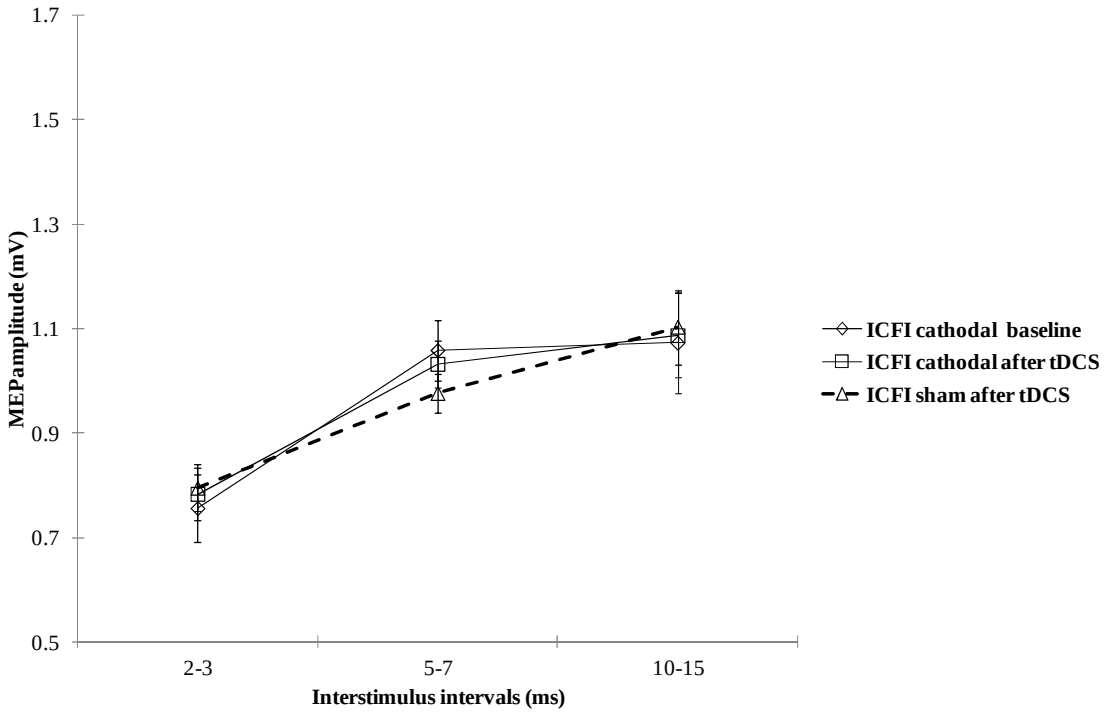


Figure 21. Motor cortex short latency intracortical inhibition/intracortical facilitation (SICI/ICF) for cathodal and sham transcranial direct current stimulation (tDCS) (experiment 2a). The conditioning stimulus (CS) was set to an intensity of 70 % of the active motor threshold (AMT). Test stimulus (TS) intensity was adjusted to evoke a motor evoked potential of about 1 mV peak-to-peak amplitude. Test stimulus amplitude was adjusted after tDCS when necessary. Interstimulus intervals (ISIs) were 2, 3, 5, 7, 10 and 15 ms (arranged in the abscissa in three groups of intervals of 2-3, 5-7 and 10-15 ms). No significant SICI/ICF alterations were present in the cathodal condition. (◊) Before tDCS condition. (€) After tDCS condition. (Δ) After sham tDCS. Error bars represent standard error of means (SEM).

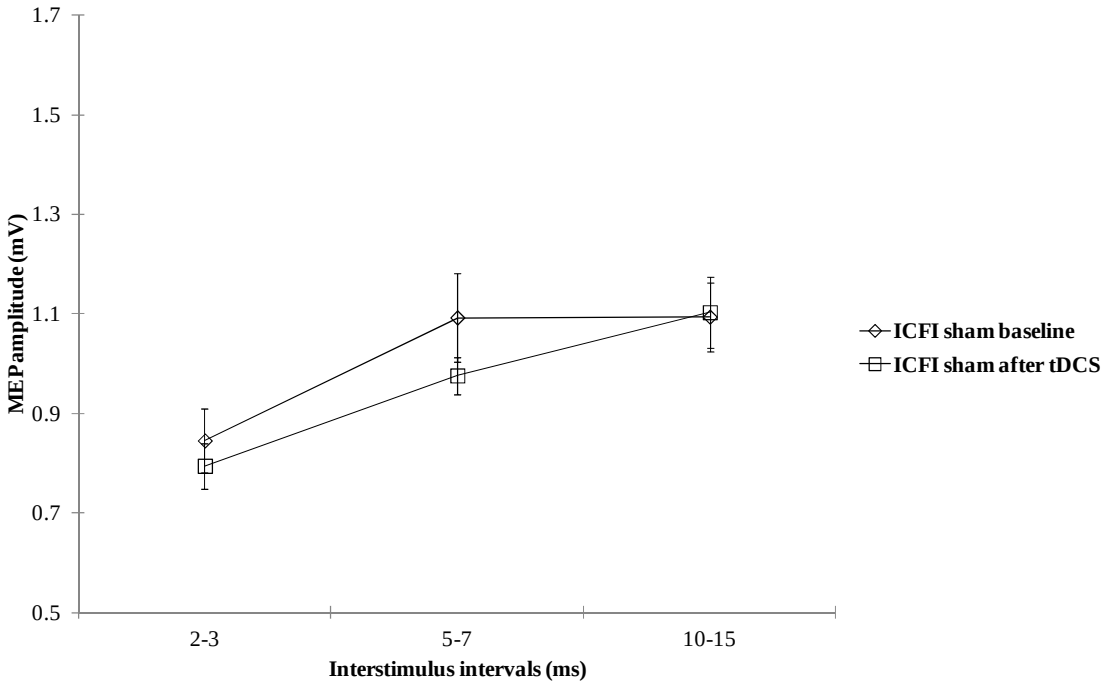


Figure 22. Motor cortex short latency intracortical inhibition/intracortical facilitation (SICI/ICF) for sham transcranial direct current stimulation (tDCS) (experiment 2a). The conditioning stimulus (CS) was set to an intensity of 70% of the active motor threshold (AMT). Test stimulus (TS) intensity was adjusted to evoke a MEP of about 1 mV peak-to-peak amplitude. Test stimulus amplitude was adjusted after tDCS when necessary. Interstimulus intervals (ISIs) were 2, 3, 5, 7, 10 and 15 ms (arranged in the abscissa in three groups of intervals of 2-3, 5-7 and 10-15 ms). Sham tDCS had no significant effect on SICI/ICF. (◊) Before tDCS condition. (∈) After tDCS condition. Error bars represent standard error of means (SEM).

4.4 EXPERIMENT 2b

The percentage of maximum stimulator output (%MSO) for motor thresholds and the absolute values for motor evoked potential baselines for all experimental sessions are shown in the Table 10.

Table 10. Baseline and motor threshold values before and after transcranial direct current stimulation (tDCS) of the experiment 2b. Data are presented as mean ± standard deviation (SD). Abbreviations: F= female; M= male; n= number of participants; RMT= resting motor threshold; SI 1mv= transcranial magnetic stimulation intensity adjusted to elicit ~ 1 mV peak to peak amplitude of motor evoked potentials (MEPs). *= Percentage of maximum stimulator output (%MSO).

				Before tDCS		
Experimental session	**n**	**Sex (M/F)**	**Age**	**SI $_{1mv}$ (%)***	**RMT (%)***	**Baseline MEP amplitude (mV)**
Anodal tDCS over P3	15	10F/5M	25.7 ± 3.4	65.8 ± 7.1	35.1 ± 4.2	1.12±0.17
Cathodal tDCS over P3	15	10F/5M	25.7 ± 3.4	66.0 ± 7.7	35.6 ± 4.5	1.03±0.13
Sham tDCS over P3	15	10F/5M	25.7 ± 3.4	66.6 ± 6.5	35.6 ± 4.0	1.04±0.15
				After tDCS		
Anodal tDCS over P3				65.2 ± 6.9	34.13 ± 4.4	1.03±0.13
Cathodal tDCS over P3				66.9 ± 7.1	36.6 ± 5.0	1.06±0.13
Sham tDCS over P3				67.0 ± 7.2	35.6 ± 3.7	1.10±0.12

The results of the ANOVA for baselines and motor threshold did not differ between experimental sessions (Table 11). The results of the ANOVA for resting motor thresholds indicates a significant interaction between stimulation and time ($P = 0.006$). Post-hoc test evidenced a significant change in the cathodal experimental session. Resting motor thresholds baseline (35.6 ± 4.5) was significantly increased ($P < 0.05$) after stimulation (36.6 ± 5.0).

Table 11. Results of the analysis of variance (ANOVA) tests of the experiment 2b. A two-way repeated-measures ANOVAs were calculated for baselines values and motor thresholds. A three-way repeated-measures ANOVAs were calculated for motor evoked potentials (MEPs). Abbreviations: d.f.= degrees of freedom; ISIs= interstimulus intervals; RMT= resting motor threshold; SICI/ICF= short latency intracortical inhibition and intracortical facilitation; tDCS= transcranial direct current stimulation; TMS= transcranial magnetic stimulation. *= $P<0.05$.

Measurement	Factor	d.f	F-value	P-value
Baseline MEP amplitude (mV)				
	Experimental session	2	0.015	0.985
	Time	1	0.399	0.538
	Experimental session x time	2	1.977	0.157
Motor threshold (MT)				
	MT stimulation	2	0.314	0.733
	MT time	1	0.516	0.484
	MT stimulation x time	2	1.567	0.226
	RMT stimulation	2	1.342	0.278
	RMT time	1	0.416	0.53
	RMT stimulation x time	2	6.115	0.006*
P3- M1 SICI/ICF				
	tDCS stimulation	2	2.994	0.69
	TMS time	1	0.613	0.447
	ISIs	2	0.457	0.638
	tDCS stimulation x TMS time	2	3.988	0.030*
	tDCS stimulation x ISIs	4	0.02	0.749
	Time x ISIs	2	0.22	0.804
	Stimulation x TMS time x ISIs	4	2.364	0.064

The repeated-measures ANOVA (Table 11) also shows a significant interaction between transcranial magnetic stimulation condition and time (P = 0.030). The results of the post-hoc tests indicate significant motor evoked potential enhancements in the anodal condition compared to baseline and sham ($P < 0.05$) for the interstimulus interval of 10-15 ms (Figure 23). In the cathodal condition, motor evoked potential amplitudes decreased compared to sham ($P < 0.05$) for the same interval (Figure 24). Motor evoked potential amplitudes tended to increase with anodal transcranial direct current stimulation and decrease with cathodal stimulation for the rest of the interstimulus intervals (Figure 25). In the sham condition, no motor evoked potential changes were

found (Figure 26). Table 12 shows the significant exact values for the post-hoc test results.

Table 12. Significant values for post hoc test of the experiment 2b. ISIs= interstimulus intervals (expressed in ms); tDCS over P3= transcranial direct current stimulation over the posterior parietal cortex region of the international 10-20 electroencephalography system.

Anodal tDCS over P3 vs Baseline	**Significance**	**Cathodal tDCS over P3 vs Baseline**	**Significance**
ISIs 10 - 15	$P = 0.005$	ISIs 10 - 15	$P = 0.026$
Anodal tDCS over P3 vs Sham	**Significance**	**Cathodal tDCS over P3 vs Sham**	**Significance**
ISIs 10 - 15	$P = 0.011$	ISIs 10 - 15	$P = 0.003$

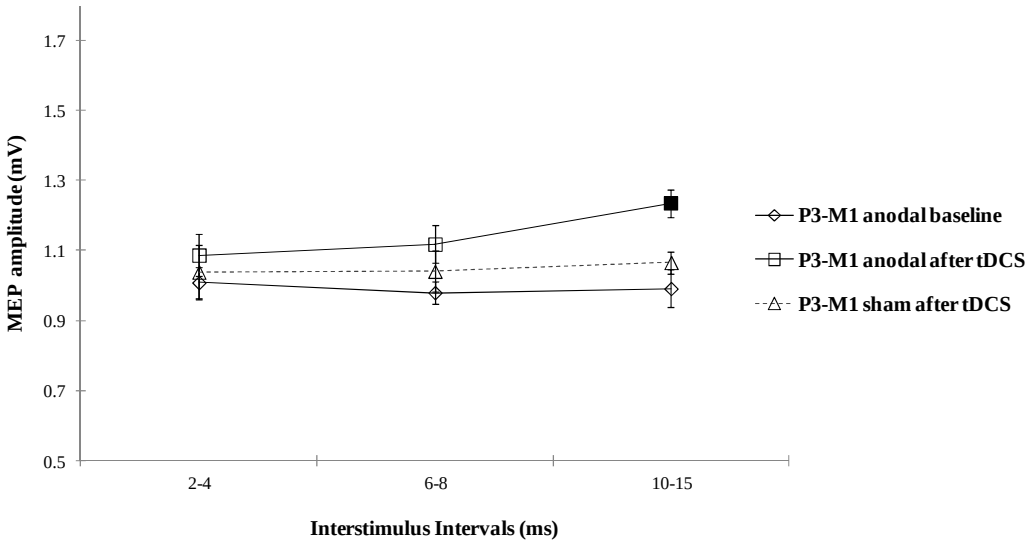


Figure 23. Parieto-motor short latency intracortical inhibition/parieto-motor intracortical facilitation (pmSICI/pmICF) after posterior parietal cortex (P3) anodal transcranial direct current stimulation (tDCS) recorded by paired-pulse parieto-motor cortex transcranial magnetic stimulation (TMS) (experiment 2b). The Conditioning stimulus (CS) was applied over P3 and the test stimulus (TS) was applied over the primary motor cortex (M1) representation of the right first dorsal interosseus (FDI) muscle (FDI). CS intensity was set to 90% of the resting motor threshold (RMT). TS was adjusted to the intensity to evoke ~1mV peak-to-peak amplitude MEP of the right FDI muscle. Interstimulus intervals (ISIs) were 2, 4, 6, 8, 10, and 15 ms (arranged in the abscissa in three groups of intervals of 2-4, 6-8 and 10-15 ms). Twenty single pulse MEPs and ten conditioned MEPs for each interstimulus interval were registered, and then anodal and sham P3 tDCS was applied. Significant pmICF changes were found for the ISIs 10-15 compared to baseline (■$P < 0.05$) and sham (*$P < 0.05$). (◊) Before tDCS condition, P3-M1 anodal tDCS baseline. ( ) After tDCS condition, P3-M1 after anodal tDCS. (Δ) After sham tDCS. (■) Anodal versus baseline significance. (*) Anodal versus sham significance. Error bars represent standard error of means (SEM).

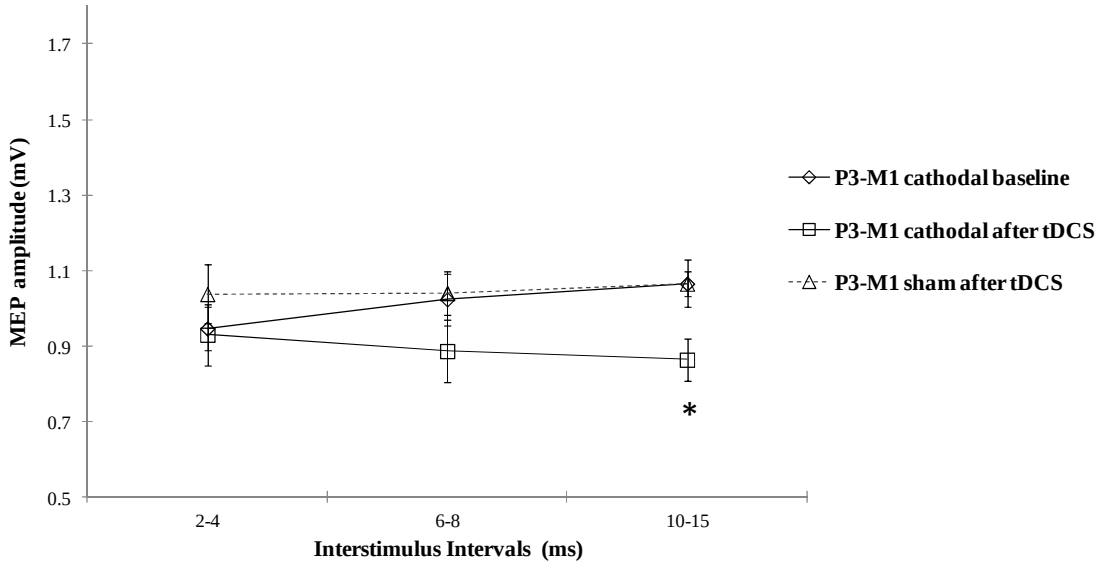


Figure 24. Parieto-motor short latency intracortical inhibition/parieto-motor intracortical facilitation (pmSICI/pmICF) after posterior parietal cortex (P3) cathodal transcranial direct current stimulation (tDCS) recorded by paired-pulse parieto-motor cortex transcranial magnetic stimulation (TMS) (experiment 2b). The Conditioning stimulus (CS) was applied over P3 and the test stimulus (TS) was applied over the primary motor cortex (M1) representation of the right first dorsal interosseus (FDI) muscle. CS intensity was set to 90% of the resting motor threshold (RMT). TS was adjusted to the intensity to evoke ~1mV peak-to-peak amplitude motor evoked potential (MEP) of the right FDI muscle. Interstimulus intervals (ISIs) were 2, 4, 6, 8, 10, and 15 ms (arranged in the abscissa in three groups of intervals of 2-4, 6-8 and 10-15 ms). Twenty single pulse MEPs and ten conditioned MEPs for each ISIs were registered, and then cathodal and sham P3 tDCS was applied. MEPs were significantly reduced after cathodal tDCS (C) for the ISIs 10-15 ms compared to sham (*P = < 0.05). (◊) Before tDCS condition, P3-M1 cathodal baseline. ( ) After tDCS condition, P3-M1 cathodal after tDCS. (Δ) After sham tDCS. (*) Cathodal versus sham significance. Error bars represent standard error of means (SEM).

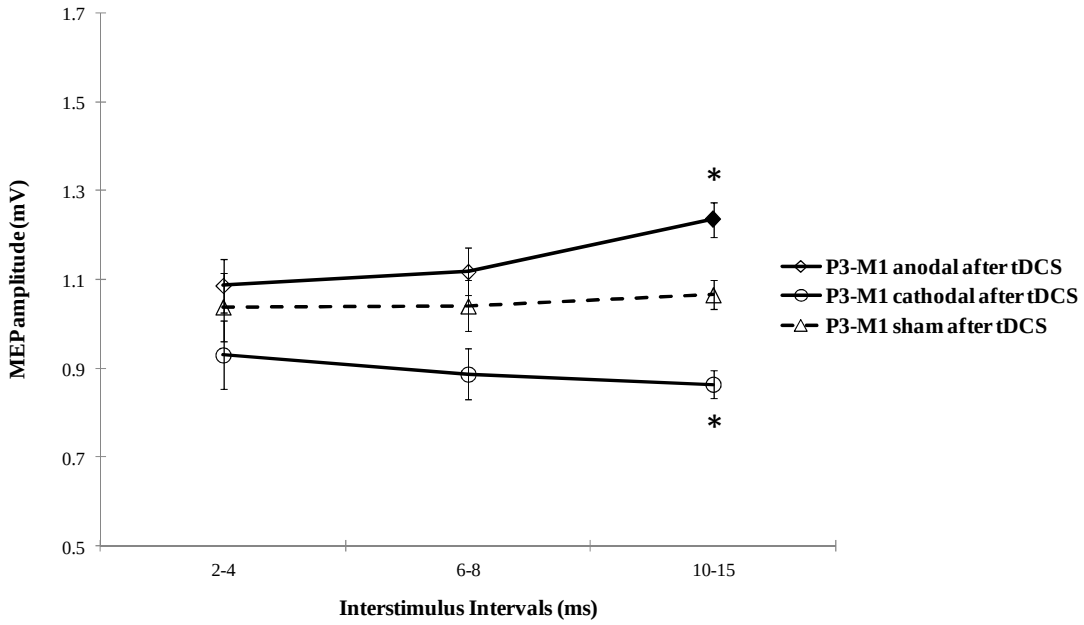


Figure 25. Parieto-motor short latency intracortical inhibition/parieto-motor intracortical facilitation (pmSICI/pmICF) after posterior parietal cortex (P3) anodal, cathodal and sham transcranial direct current stimulation (tDCS) recorded by paired-pulse parieto-motor cortex transcranial magnetic stimulation (TMS) (experiment 2b). Motor evoked potential (MEP) amplitudes tended to increase with anodal tDCS and decrease with cathodal tDCS, with significant differences in the interval of 10-15 ms. (◊) Posterior parietal cortex (P3)-primary motor cortex (M1) connectivity changes after anodal tDCS condition. (o) P3-M1connectivity changes after cathodal tDCS condition. (Δ) P3)-M1 connectivity after sham transcranial direct current stimulation condition. (*) tDCS versus sham significance. (♦) Anodal versus baseline significance (baseline is not shown in this figure). Error bars represent standard error of means (SEM).

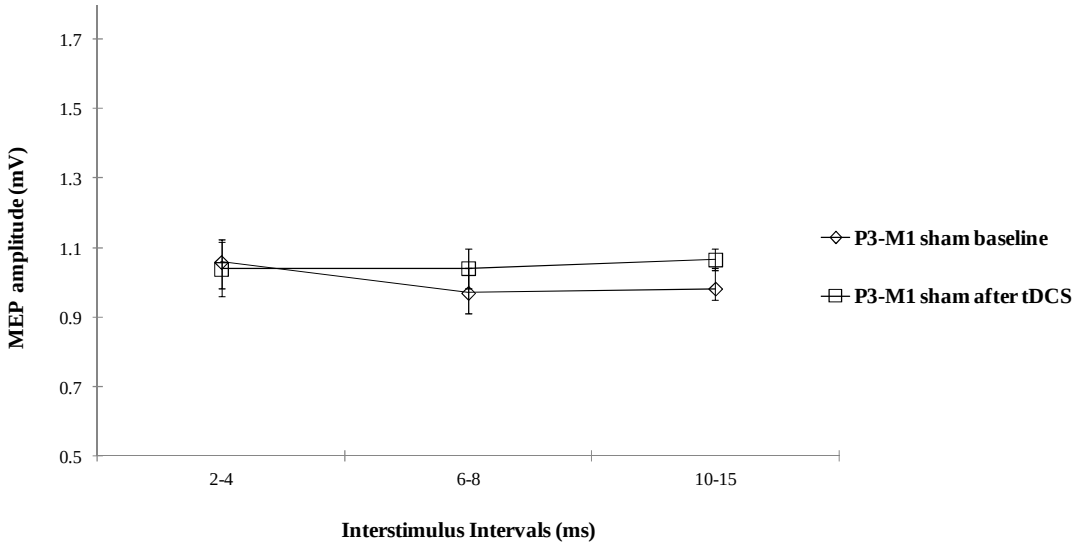


Figure 26. Parieto-motor short latency intracortical inhibition/parieto-motor intracortical facilitation (pmSICI/pmICF) after posterior parietal cortex (P3) sham transcranial direct current stimulation (tDCS) recorded by paired-pulse parieto-motor cortex transcranial magnetic stimulation (TMS) (experiment 2b). The conditioning stimulus (CS) was applied over posterior parietal cortex (P3) and the test stimulus (TS) was applied over the primary motor cortex (M1) representation of the right first dorsal interosseus muscle (FDI). CS intensity was set to 90% of the resting motor threshold (RMT). TS was adjusted to the intensity to evoke ~1mV peak-to-peak amplitude MEP of the right FDI. Interstimulus intervals (ISIs) were 2, 4, 6, 8, 10, and 15 ms (arranged in the abscissa in three groups of intervals of 2-4, 6-8 and 10-15 ms). Twenty single pulse MEPs and ten conditioned MEP for each ISI were registered, and then sham P3 tDCS was applied. Sham tDCS did not result in any significant change of SICI/ICF. (◊) Before tDCS condition, P3-M1 sham baseline. (∈) After tDCS condition, P3-M1 sham after tDCS. Error bars represent standard error of means (SEM).

5. DISCUSSION

Transcranial magnetic stimulation is a renowned tool used to record changes in the plasticity of the brain (Pascual-Leone et al., 1999; Pascual Leone et al., 2011; Freitas et al., 2013). On the other hand, it is known that some specific stimuli produce neuroplastic changes in primary motor cortex (Borgomaneri, 2013; Pisoni et al., 2014). In a recent study, Vicario et al. (2014) interestingly reported differences in the motor evoked potentials registered in two different muscles in response to the same stimulus. The amplitude of the tongue motor evoked potentials was increased when visual cues related to cigarette were observed by smokers, but there was no change in the excitability of the carpi radialis muscle. The authors propose that this change in the excitability of the two different muscles recorded by the motor evoked potentials could be related to the differential involvement of the rewarded system. Specific visual (Fadiga et al., 1995, 2005; Mattiasi et al., 2014; Vicario et al., 2014) or auditory stimuli (Flöel et al., 2003; Sowman et al., 2014), as well as the transcranial magnetic stimulation conditioning stimulus (Kujirai et al., 1993; Ziemann et al., 1996; Rothwell, 1999), have shown the faculty to change the motor cortex excitability. Thus, this tool makes it possible, for example, to research intracortical inhibition and facilitation, and facilitatory and inhibitory interregional connectivity.

Transcranial direct current stimulation is a non-invasive brain stimulation technique which has reached a renewed interest since the studies of Nitsche and Paulus (2000). They reported changes in the motor cortex excitability measured by transcranial magnetic stimulation when transcranial direct current stimulation was applied over the motor cortex. Since then, the use of this tool to research the cortical excitability changes has markedly increased. In contrast to other stimuli, it has the property to keep the cortical excitability changes for an hour. Because the duration of this effect, some

researchers are interested in the possibility of using transcranial direct current stimulation for rehabilitation in neurological disorders (Fregni et al., 2005a; Kumru et al., 2013; Pereira et al., 2013), or even to improve learning abilities (Nitsche et al., 2003d; Fregni et al., 2005b; Peña-Gómez et al., 2011; Pavlova et al., 2014).

The combination of these two non-invasive brain stimulation tools allows extend the research about the neuroplastic changes to the study of the interregional connectivity in the human brain. Indeed, some research using both techniques has already shown this possibility (Boros et al., 2008). Our general results also point to an interregional connectivity, specifically between the posterior parietal cortex region of the international 10-20 electroencephalography system and the primary motor cortex.

The data of this thesis show that P3 transcranial direct current stimulation induces polarity-dependent primary motor cortex excitability changes. Anodal stimulation of P3 enhances motor evoked potential elicited by single-pulse transcranial magnetic stimulation over the primary motor cortex, intracortical changes at 5-7 ms and 10-15 ms interstimulus intervals in the primary motor cortex, and the parieto-motor cortex intracortical excitability at interstimulus intervals 10-15 ms. Cathodal stimulation of the posterior parietal cortex region reduces motor evoked potential elicited by single-pulse transcranial magnetic stimulation in primary motor cortex, and parieto-motor cortex intracortical excitability at interstimulus interval 10-15 ms. Cortico-spinal excitability alterations remain up to 120 min after stimulation. Anodal or cathodal transcranial direct current stimulation over 3 cm posterior or lateral to P3 does not affect the primary motor cortex excitability consistently.

5.1 EXPERIMENT 1a

Primary motor cortex excitability changes induced by parietal transcranial direct current stimulation

The results of this experiment suggest that parietal transcranial direct current stimulation changes motor cortex excitability. In particular, anodal current stimulation over P3 increased the excitability of the primary motor cortex. This effect was present for one hour after stimulation. Cathodal stimulation over P3 reduced the primary motor cortex excitability, but this effect was somewhat weaker. Sham transcranial direct current stimulation had no effect on the excitability of the primary motor cortex. The direction of the effect of this stimulation at P3 is the same as applying it over the primary motor cortex, however the duration of the effects seem to be longer-lasting than those of roughly comparable anodal current stimulation over the primary motor cortex, whereas the after effects of cathodal stimulation recorded over a period of 60 min are considerably shorter when compared with 9 min (Nitsche and Paulus, 2001, Nitsche et al., 2003a) or 18 min of the motor cortex stimulation (Monte-Silva et al., 2010). Thus the results deliver evidence that it is possible to induce neuroplastic changes of the motor cortex excitability by applying stimulation over connected cortical areas. However, the results of this experiment on its own do not allow to conclude if this is a connectivity-driven effect or due to a current spread to the primary motor cortex. Nevertheless, the specific changes of excitability in the motor cortex after stimulation and the fact that there was no effect on the input-output curve make difficult to support this possibility. Two different areas near to P3 were stimulated in the next experiment in order to explore the spatial specificity of the effect. Since anodal stimulation over P3 did cause changes in the excitability of primary motor cortex lasting up 60 min after

transcranial direct current stimulation, the duration of the effect in the next experiment was explored for two hours.

5.2 EXPERIMENT 1b

Spatial specificity of the effects of parietal transcranial direct current stimulation on primary motor cortex excitability

The stimulation of areas situated near to P3 had no consistent effect on primary motor cortex excitability. Anodal and cathodal direct current stimulation over 3 cm lateral to P3 did not cause any change of the primary motor cortex excitability. Both stimulations applied 3 cm posterior to P3 induced minor, but significant, excitability changes only 10 and 60 min after stimulation. In contrast, anodal stimulation over P3 increased the primary motor cortex excitability for 120 min after the stimulation, and cathodal stimulation reduced the primary motor cortex excitability, and this effect recorded over a period of 120 min lasted from 5 min to two hours after the stimulation. Thus, the neuroplastic changes induced by transcranial direct current stimulation occur grossly when the stimulation is selectively applied over P3, which is compatible with a primarily cortico-cortical connectivity effect (Koch et al., 2008; Karabanov et al., 2012, 2013). Since the alterations of the excitability of the motor cortex persist for at least two hours, it is reasonable to think that the plasticity induced by transcranial direct current stimulation over parietal cortex might have important clinical applications in motor disorders resulting from brain damage. Consequently, it could definitely be a primary objetive to research in the future.

Interestingly, in this experiment the cathodal effects seem to be more robust as those in the experiment 1a, which might be due to interindividual differences. In this sense, in order to explore the combined data of the experiments 1a and 1b and compare them with those of each separated study, and evaluate the differences between anodal and cathodal effects, we analyzed the motor evoked potential amplitudes from both experiments together. At 0 min, 30 min and 60 min the aftereffects recorded in the anodal current stimulation condition were more intense compared to the baseline than in the case of the cathodal current stimulation, even considering the higher variability found in the anodal stimulation. These data confirm the differential effects on the cortical excitability resulting from the different currents applied. Taken together, these data again suggest a specific posterior parietal cortex-primary motor cortex connectivity effect induced by transcranial direct current stimulation, which depend on the specific current used. This cortico-cortical effect was again suggested from the results of the following experiments.

5.3 EXPERIMENT 2a

Short-latency intracortical inhibition/intracortical facilitation changes induced by parietal transcranial direct current stimulation

Anodal transcranial direct current stimulation over P3 resulted in excitatory effects at the neutral (5-7 ms) and facilitatory (10-15 ms) interstimulus intervals. In contrast, cathodal stimulation over P3 did not alter the primary motor cortex intracortical excitability. Therefore, anodal parietal stimulation can activate pathways of intracortical facilitation of motor cortex neurons. Another study has shown similar short latency intracortical inhibition and intracortical facilitation changes after premotor cortex

transcranial direct current stimulation (Boros et al., 2008), although in this case the pattern of induced plasticity was not exactly the same. In that study, anodal stimulation of the premotor cortex enhanced the intracortical facilitation of the primary motor cortex and reduced the intracortical inhibition, but had no impact in the motor evoked potential amplitudes elicited by single pulse transcranial magnetic stimulation. The reason for these discernible effects is unclear at present, but might be related to different location of the terminations of parietal and premotor afferents on primary motor cortex neurons (Godschalk et al., 1984; Leichnetz, 1986; Tokuno and Nambu, 2000). Alternatively, it cannot be excluded that current spread from the parietal electrode contributed to the effects. But certainly our results are basically consistent with a specific facilitatory effect on parieto-motor connectivity. The fact that there were no alterations at interstimulus interval 2-3 ms (which are related to intracortical inhibition) after any stimulation condition, and the changes in the excitability were produced by anodal stimulation at the no-inhibitory interstimulus interval of 5-7 ms and 10-15 ms, suggest that the posterior parietal cortex-primary motor cortex connectivity can be modulated more easily inducing intracortical facilitation via anodal current stimulation. These results are compatible with those reported by Koch et al. (2007).

5.4 EXPERIMENT 2b

Parietal-motor cortical connectivity changes induced by parietal transcranial direct current stimulation

Anodal and cathodal P3 induced primary motor cortex excitability changes at the facilitatory interstimulus interval (10-15 ms). Specifically, anodal current stimulation

increased the parieto-motor cortex excitability and cathodal current stimulation decreased it. No excitability changes were found at the remaining interstimulus interval (2-4 and 6-8 ms). Previous studies using twin coil transcranial magnetic stimulation describe motor cortex excitability alterations when a conditioning stimulus was applied over the parietal cortex (Koch et al., 2007; Koch and Rothwell, 2009; Karabanov et al., 2012, 2013). The alterations of these connections induced by transcranial direct current stimulation support the idea of polarity-specific neuroplastic effects of parietal transcranial direct current stimulation on parietal-motor connections.

The results of the present study show that direct current stimulation targeted at the left posterior parietal cortex induces neuroplastic changes of the excitability of the ipsilateral primary motor cortex, as explored by transcranial magnetic stimulation. Anodal stimulation of the posterior parietal cortex enhanced the motor cortex cortico-spinal excitability, while cathodal stimulation had antagonistic effects. These results could be partially explained by a physical current spread (Faria et al., 2011; Edwards et al., 2013), although not completely because P3 transcranial direct current stimulation had a specific impact on the motor cortex excitability, which depended on the exact position of the stimulation electrodes. Slight deviations of electrode positions resulted in clear alterations of the transcranial direct current stimulation effects, which is difficult to explain by physical current spread alone. In accordance, the results of experiments 2a and 2b are well compatible with a predominating connectivity effect, because in these studies the parietal transcranial direct current stimulation had a specific effect on the motor cortex intracortical excitability and parietal-motor cortex excitability. In the case of the intrinsic motor cortical excitability, again a current spread effect cannot be completely ruled out, although these results are explained more easily due to a selective

influence of parietal transcranial direct current stimulation on intracortical facilitatory networks between the posterior parietal and primary motor cortex. This hypothesis was finally confirmed in experiment 2b. Using a twin coil transcranial magnetic stimulation protocol, anodal P3 transcranial direct current stimulation induced a facilitatory effect on the motor cortex, whereas cathodal current stimulation reduced the parieto-motor cortex facilitation.

The impact of transcranial direct current stimulation over the parietal cortex on motor cortex excitability is consistent with the results of previous studies in humans, and animals, in which connections between the motor and posterior parietal cortices were demonstrated (Inman et al., 2012). In humans, this anatomical connection has also been explored by transcranial magnetic stimulation, showing specific regions of the parietal cortex connecting to the motor cortex (Karabanov et al., 2012, 2013). Since parietal and primary motor areas are functionally interconnected during motor learning (Koch et al., 2007), it is likely that the connections between these structures undergo plastic changes, such as it is the case for prefrontal networks (Esslinger et al., 2014). Targeting these plastic alterations would reveal an additional tool for modulating interregional plasticity in motor function. Modulation of connectivity may have implications for motor learning and motor rehabilitation processes. Underscoring the possible relevance of interregional plasticity for motor performance, some transcranial magnetic stimulation studies have shown connectivity changes associated to simple motor task performance (Hortobágyi et al., 2011). More support for this possibility could be found through the study of different motor learning process.

5.5 FUTURE REMARKS

Accordingly to present results, our next experiments are aimed to evaluate the role of the non-invasive parietal stimulation on learning motor. In this regard, the data reported in this thesis will be completed with our new research project designed to know the behavioral effects of the transcranial direct current stimulation applied over the posterior parietal cortex by recording the performance in motor learning tasks.

In this sense, serial reaction time task and reaction time task, described by Nissen and Bullemer (1987), have been used to study possible changes in the motor learning after non-invasive brain stimulation. For example, Kuo et al (2008) have used a serial reaction time task version (for details of the specific procedure see Annex I) to study the effect of the motor cortex transcranial direct current stimulation on motor learning. From their results they conclude that the effect of this type of stimulation on motor learning could be more optimal if the stimulation is applied just during the task execution.

In another research, Nitsche et al. (2010) studied the influence of the premotor cortex transcranial direct current stimulation during rapid eye movements (REM) sleep phase on a similar sequence learning task. They found that anodal current stimulation applied over the premotor cortex during REM sleep was able to decrease the reaction time in the serial reaction time task. In contrast, cathodal stimulation had no significant effect on the task.

Thus, in our next project, the use of this task could be a reliable tool to study the effect of the posterior parietal cortex transcranial direct current stimulation on implicit motor learning. By this behavioral procedure, we could evaluate the functional correlate

of the neuroplastic changes induced on the P3-M1 connectivity by transcranial direct current stimulation. The specific aim is to explore the effect of anodal P3 transcranial direct current stimulation versus anodal primary motor cortex in two different phases of learning: during the performance of a serial reaction time task (acquisition phase) (according to the Kuo et al., 2008's proposal), and in the consolidation of learning, several hours after the execution of the task (see Annex II for details of the procedure). Thus, this research project could help to understand the involvement of the P3-M1 connectivity in motor learning.

On the other hand, the possibility of modifying the motor response by non-invasive brain stimulation could contribute to design new strategies for clinical interventions in patients with motor or sensory neurological disorders such as neglect, apraxia, phantom limb (Bolognini et al., 2013), or neuropsychological disorders as dyscalculia (Hauser et al., 2013; Luculano and Cohen, 2014).

In summary, the results of the current studies in this thesis deliver evidence for motor cortex plasticity induced by parietal transcranial direct current stimulation. Knowledge about plasticity of posterior parietal cortex-primary motor cortex connectivity could help to identify mechanisms of motor learning to a larger extent. The data fit well with anatomical and functional connectivity between both areas. Nevertheless, the final confirmation that this effect is connectivity-driven and its functional relevance need to be obtained in future studies which will allow to test the impact of cortico-cortical plasticity effects induced by non-invasive brain stimulation on different motor learning tasks. This knowledge may be useful in clarifying specifically how the brain changes and plasticity induced by non-invasive brain stimulation

protocols can modulate the motor performance in healthy people, or even with neurological motor disorders.

6. CONCLUSIONS

The present Doctoral Thesis was primarily focused to the study of specific plasticity of the parietal-motor connectivity and their functional importance. Non-invasive brain stimulation protocols were used to reach the specific proposed objectives. The main findings of this study are the following:

1. Posterior parietal (P3) transcranial direct current stimulation induces polarity-dependent primary motor cortex excitability changes.
2. P3 anodal stimulation enhances the primary motor cortex cortico-spinal excitability, and increases the primary motor cortex intracortical facilitation and parieto-motor cortex intracortical facilitation.
3. P3 cathodal stimulation reduces the primary motor cortex cortico-spinal excitability, and the parieto-motor cortex intracortical excitability.
4. Anodal or cathodal P3 transcranial direct current stimulation has no effect on the primary motor cortex intracortical inhibition.
5. Transcranial direct current stimulation over 3 cm lateral or posterior to P3 has no significant effect on the primary motor cortex excitability.
6. The cortico-spinal excitability changes induced by transcranial direct current stimulation remain at least 120 min after stimulation.
7. These parieto-motor cortex effects are at least partially compatible with a connectivity-driven effect.

7. REFERENCES

REFERENCES

Albert DJ (1966) The effects of polarizing currents on the consolidation of learning. Neuropsychologia 4: 65-77.

Antal A, Nitsche MA, Kincses TZ, Kruse W, Hoffmann KP, Paulus W (2004) Facilitation of visuo-motor learning by transcranial direct current stimulation of the motor and extrastriate visual areas in humans. European Journal of Neuroscience 19(10): 2888-92.

Aruta, A (2011) Shocking waves at the museum: The Bini–Cerletti Electro-shock apparatus. Medical History 55: 407–12.

Bartrés-Faz D, Tormos JM, Junqué C, Pascual-Leone A (2000) Transcranial magnetic stimulation: contribution to psychiatry and to the study of brain-behavior relationship Actas Esp Psiquiatr 28(2):130-6.

Bashir S, Perez JM, Horvath JC, Pena-Gomez C, Vernet M, Capia A, Alonso-Alonso M, Pascual-Leone A (2014) Differential effects of motor cortical excitability and plasticity in young and old individuals: a Transcranial Magnetic Stimulation (TMS) study. Front Aging Neurosci 6:111. doi: 10.3389/fnagi.2014.00111.

Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA (2013) Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 591(7): 1987-2000.

Bolognini N, Olgiati E, Maravita A, Ferraro F, Fregni F (2013) Motor and parietal cortex stimulation for phantom limb pain and sensations. Pain 154(8): 1274-80.

Borgomaneri S, Gazzola V, Avenanti A (2013) Temporal dynamics of motor cortex excitability during perception of natural emotional scenes. Soc Cogn Affect Neurosci. PMID: 23945998.

Boros K, Poreisz C, Münchau A, Paulus W, Nitsche MA (2008) Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. European Journal of Neuroscience 27(5): 1292-300.

Burman KJ, Bakola S, Richardson KE, Reser DH, Rosa MG (2014) Patterns of cortical input to the primary motor area in the marmoset monkey. J Comp Neurol 522(4): 811-43.

Caparros-Lefebvre D, Blond S, Vermersch P, Pécheux N, Guieu JD, Petit H (1993) Chronic thalamic stimulation improves tremor and levodopa induced dyskinesias in Parkinson's disease. J Neurol Neurosurg Psychiatry 56(3): 268-73.

Civardi C, Cantello R, Asselman P Rothwell JC (2001) Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. Neuroimage 14(6): 1444-53.

Chao CC, Karabanov AN, Paine R, Carolina de Campos A, Kukke SN, Wu T, Wang H, Hallett M (2013) Induction of motor associative plasticity in the posterior parietal cortex-primary motor network. Cereb Cortex PMID: 23968834.

Chen WH, Mima T, Siebner HR, Oga T, Hara H, Satow T, Begum T, Nagamine T, Shibasaki H. (2003) Low-frequency rTMS over lateral premotor cortex induces lasting changes in regional activation and functional coupling of cortical motor areas. Clinical Neurophysiology 114: 1628-37.

Convento S, Bolognini N, Fusaro M, Lollo F, Vallar G (2014) Neuromodulation of parietal and motor activity affects motor planning and execution. Cortex 28(57C): 51-9.

Coxon JP, Peat NM, Byblow WD (2014) Primary motor cortex disinhibition during motor skill learning. J Neurophysiol 112(1):156-64.

Cuypers K, Leenus DJ, van den Berg FE, Nitsche MA, Thijs H, Wenderoth N, Meesen RL (2013) Is motor learning mediated by tDCS intensity? PLoS One 8(6):e67344.

Dejerine J (1895) Anatomie des centres nerveux. Paris: Reuff et Cie.

Edwards D, Cortes M, Datta A, Minhas P, Wassermann EM and Bikson M (2013) Physiological and modeling evidence for focal transcranial electrical brain stimulation in humans: A basis for high-definition tDCS. Neuroimage 74: 266-75.

Esslinger C, Schüler N, Sauer C, Gass D, Mier D, Braun U, Ochs E, Schulze TG, Rietschel M, Kirsch P, Meyer-Lindenberg A (2014) Induction and quantification of prefrontal cortical network plasticity using 5 Hz rTMS and fMRI. Hum Brain Mapp 35: 140-51.

Fadiga L, Fogassi G, Pavesi G, Rizzolatti G (1995) Motor facilitation during action observation: a magnetic stimulation study. J Neurophysiol 73(6): 2608-11.

Fadiga L, Craighero L, Olivier E (2005) Human motor cortex excitability during perception of others' action. Curr Opin Neurobiol 15(2): 213-18.

Faria P, Hallett M, Miranda PC (2011) A finite element analysis of the effect of electrode area and inter-electrode distance on the spatial distribution of the current density in tDCS. J Neural Eng 8(6):066017. doi: 10.1088/1741-2560/8/6/066017.

Feurra M, Bianco G, Polizzotto NR, Innocenti I, Rossi A, Rossi S (2011) Cortico-cortical connectivity between right parietal and bilateral primary motor cortices during imagined and observed actions: a combined TMS/tDCS study. Front Neural Circuits 5(10): 1-9.

Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD (1992) Interhemispheric inhibition of the human motor cortex. J Physiol 453: 525-46.

Flöel A, Ellger T, Breitenstein C, Knecht S (2003) Language perception activates the hand motor cortex: implications for motor theories of speech perception. Eur J Neurosci. 18(3): 704-8.

Fregni F, Boggio PS, Mansur CG, Wagner T, Ferreira MJ, Lima MC, Rigonatti SP, Marcolin MA, Freedman SD, Nitsche MA, Pascual-Leone A (2005a) Transcranial direct current stimulation of the unaffected hempisphere in stroke patients. Neuroreport 16(14): 1551-5.

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, Marcolin MA, Rigonatti SP, Silva MT, Paulus W, Pascual-Leone A (2005b) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 166(1): 23-30.

Freitas C, Farzan F, Pascual-Leone A (2013) Assessing brain plasticity across the lifespan with transcranial magnetic stimulation: why, how, and what is the ultimate goal? Front. Neurosci 7(42): 1-17.

Frey SH and Gerry VE (2006) Modulation of neural activity during observational learning of actions and their sequential orders. J Neurosci 26(51): 13194-201.

Furubayashi T, Terao Y, Arai N, Okabe S, Mochizuki H, Hanajima R, Hamada M, Yugeta A, Inomata-Terada S, Ugawa Y (2008) Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. Exp Brain Res 185: 279–86.

Gharbawie OA, Stepniewska I, Qi H, Kaas JH (2012) Multiple parietal-frontal pathways mediate grasping in macaque monkeys. J Neurosci 31(32): 11660-77.

Godschalk M, Lemon RN, Kuypers HG Ronday HK (1984) Cortical afferents and efferents of monkey postarcuate area: an anatomical and electrophysiological study. Exp Brain Res 56: 410-24.

Grundey J, Freznosa S, Klinker F, Lang N, Paulus W, Nitsche MA. (2013) Cortical excitability in smoking and not smoking individuals with and without nicotine. Psychopharmacology 229(4): 653-64.

Hasan MT, Hernández-González S, Dogbevia G, Treviño M, Bertocchi I, Gruart A, Delgado-García JM (2013) Role of motor cortex NMDA receptors in learning-dependent synaptic plasticity of behaving mice. Nat Commun 4:2258 doi: 10.1038/ncomms3258.

Hauser TU, Rotzer S, Grabner RH, Mérillat S, Jäncke L (2013) Enhancing performance in numerical magnitude processing and mental arithmetic using transcranial Direct Current Stimulation (tDCS). Front Hum Neurosci 6;7:244. doi: 10.3389/fnhum.2013.00244.

Herwig U, Satrapi P, Schonfeldt-Lecuona C (2003) Using the international 10–20 EEG system for positioning of transcranial magnetic stimulation. Brain Topogr 16: 95-9.

Honda M, Deiber MP, Ibáñez V, Pascual-Leone A, Zhuang P, Hallett M (1998) Dynamic cortical involvement in implicit and explicit motor sequence learning. A PET study. Brain 121(11): 2159-73.

Hortobágyi T, Richardson SP, Lomarev M, Shamim E, Meunier S, Russman H, Dang N, Hallett M (2011) Interhemispheric plasticity in humans. Med Sci Sports Exerc 43: 1188-99.

Hubel DH and Wiesel TN (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J. Neurophysiol 28: 1029–40.

Hubel DH and Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J.Physiol 206: 419–36.

Hubel DH, Wiesel TN (1980) The development of ocular dominance columns in normal and visually deprived monkeys. J Comp Neurol 191(1):1-51.

Inman CS, James GA, Hamann S, Rajendra JK, Pagnoni G, Butler AJ (2012) Altered resting-state effective connectivity of fronto-parietal motor control systems on the primary motor network following stroke. Neuroimage 59: 227-37.

Karabanov A, Jin SH, Joutsen A, Poston B, Aizen J, Ellenstein A (2012) Timing-dependent modulation of the posterior parietal cortex-primary motor cortex pathway by sensorimotor training J Neurophysiol 107(11):3190-9.

Karabanov AN, Chi-Chao C, Paine R, Hallett M (2013) Mapping different intra-hemispheric parietal-motor networks using twin coil TMS. Brain Stimul 6: 384-9.

Khedr EM, Ahmed MA, Hamdy A, Shawky OA. (2011) Cortical excitability of amyotrophic lateral sclerosis: transcranial magnetic stimulation study. Neurophysiol Clin 41(2): 73-9.

Knotkova H, Nitsche MA, Cruciani RA (2013) Putative physiological mechanisms underlying tDCS analgesic effects. Front Hum Neurosci 26;7:628. doi: 10.3389/fnhum.2013.00628.

Koch G, Fernandez M, Cheeran B, Ruge D, Schippling S, Caltagirone C, and Rothwell JC (2007) Focal stimulation of the posterior parietal cortex increases the excitability of the ipsilateral motor cortex. J Neurosci 27(25): 6815-22.

Koch G, Fernandez M, Cheeran B, Schippling S, Caltagirone C, Driver J, Rothwell JC (2008) Functional interplay between posterior parietal and ipsilateral motor cortex revealed by twin-coil transcranial magnetic stimulation during reach planning toward contralateral space. J Neurosci 28(23): 5944-53.

Koch G, Ponzo V, Di Lorenzo F, Caltagirone C, Veniero D (2013) Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections. J Neurosci 33(23):9725-33. doi: 10.1523/JNEUROSCI.4988-12.2013.

Koch G, Rothwell JC (2009) TMS investigations into the task-dependent functional interplay between human posterior parietal and motor cortex. Behav Brain Res 202: 147-52.

Krause V, Bashir S, Pollok B, Caipa A, Schnitzler A, Pascual-Leone A (2012) 1Hz rTMS of the left posterior parietal cortex (PPC) modifies sensorimotor timing. Neuropsychologia 50: 3729-35.

Krauss JK, Jankovic J (1996) Surgical treatment of Parkinson's disease. Am Fam Physician 54(5): 1621-9.

Kuhl PK, Williams KA, Lacerda F, Stevens KN, Lindblom B. (1992) Linguistic experience alters phonetic perception in infants by 6 months of age. Science 255(5044): 606-8.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471: 501-19.

Kumru H, Soler D, Vidal J, Navarro X, Tormos JM, Pascual-Leone A, Valls-Sole J (2013) The effects of transcranial direct current stimulation with visual illusion in neuropathic pain due to spinal cord injury: an evoked potentials and quantitative thermal testing study. Eur J Pain 17(1): 55-66.

Kuo MF, Paulus W, Nitsche MA. (2014) Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. Neuroimage 15(85): 948-60.

Kuo MF, Unger M, Liebetanz D, Lang N, Tergau F, Paulus W, Nitsche MA (2008) Limited impact of homeostatic plasticity on motor learning in humans. Neuropsychologia 46(8): 2122-8.

Lang N, Hasan A, Sueske E, Paulus W, Nitsche MA (2008) Cortical hypoexcitability in chronic smokers? A transcranial magnetic stimulation study. Neuropsychopharmacology 33(10): 2517-23.

Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN (2004) Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Experimental Brain Research 156: 439-43.

Leichnetz GR (1986) Afferent and efferent connections of the dorsolateral precentral gyrus (area 4, hand/arm region) in the macaque monkey, with comparisons to area 8. J Comp Neurol 254: 460-92.

Li P, Legault J, Litcofsky KA (2014) Neuroplasticity as a function of second language learning: Anatomical changes in the human brain. Cortex. doi: 10.1016/j.cortex.2014.05.001.

Luculano T, Cohen FR (2014) Preliminary evidence for performance enhancement following parietal lobe in Developmental Dyscalculia. Front Hum Neurosci 8:38. doi: 10.3389/fnhum.2014.00038.

Luria A (1966) Higher Cortical Function in Man. New York: Basic Books.

Männel C, Friederici AD (2013) Accentuate or repeat? Brain signatures of developmental periods in infant word recognition. Cortex 49(10): 2788-98.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44(1): 5-21.

Márquez-Ruiz J, Leal-Campanario R, Sánchez-Campusano R, Molaee-Ardekani, B, Wendling F, Miranda PC, Ruffini G, Gruart A, Delgado-García, JM (2012)

Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits. Proc Natl Acad Sci U S A. 109(17): 6710-15.

Mattiassi AD, Mele S, Ticini LF, Urgesi C (2014) Conscious and unconscious representations of observed actions in the human motor system. J Cogn Neurosci. 26(9):2028-41.

Medina J, Beauvais J, Datta A, Bikson M, Coslett BH, Hamilton RH (2013) Transcranial direct current stimulation accelerates allocentric target detection. Brain Stimul 6(3): 433–9.

Medina FJ, Túnez I. (2013) Mechanisms and pathways underlying the therapeutic effect of transcranial magnetic stimulation. Rev Neurosci; 24(5):507-25.

Meyer BU, Röricht S, Gräfin von Einsiedel H, Kruggel F, Weindl A (1995) Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum. Brain 118(2):429-40.

Molaee-Ardekani B, Márquez-Ruiz J, Merlet I, Leal-Campanario R, Gruart A, Sánchez-Campusano R, Birot G, Ruffini G, Delgado-García JM, Wendling F (2013) Effects of transcranial Direct Current Stimulation (tDCS) on cortical activity: a computational modeling study. Brain Stimul 6(1): 25-39.

Monte-Silva K, Kuo M-F, Liebetanz D, Paulus W, Nitsche MA (2010) Shaping the Optimal Repetition Interval for Cathodal Transcranial Direct Current Stimulation (tDCS). J Neurophysiol 103: 1735-40.

Mori S, Oishi K, Faria V A. (2009) White matter atlases based on diffusion tensor imaging. Curr Opin Neurol 22(4): 362-69.

Nissen M J, Bullemer P (1987) Attentional requirements of learning: Evidence from performance measures. Cognitive Psychology 19, 1-32.

Nitsche, M.A. (2002) Transcranial direct current stimulation: a new treatment for depression? Bipolar Disorders 4(1): 98-99.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A (2008) Transcranial direct current stimulation: State of the art. Brain Stimul 1: 206-23.

Nitsche MA, Doemkes S, Karakose T, Antal A, Liebetanz D, Lang N, Tergau F, Paulus W (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex J Neurophysiol, 97(4): 3109–17.

Nitsche MA, Grundey J, Liebetanz D, Lang N, Tergau F, Paulus W (2004) Catecholaminergic consolidation of motor cortical neuroplasticity in humans. Cerebral Cortex 14(11): 1240-5.

Nitsche MA, Jakoubkova M, Thirugnanasambandam N, Schmalfuss L, Hullemann S, Sonka K, Paulus W, Trenkwalder C, Happe S (2010) Contribution of the premotor cortex to consolidation of motor sequence learning in humans during sleep. Journal of Neurophysiology 104(5): 2603-14.

Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W (2003a) A Modulation of cortical excitability by weak direct current stimulation: technical, safety and functional aspects. Supplements to Clinical Neurophysiology, 56: 255-76.

Nitsche M.A, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W (2003b) Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clinical Neurophysiology, 114(11): 2220-22.

Nitsche, MA, Liebetanz D, Tergau F, Paulus W (2002) Modulation of cortical excitability by transcranial direct current stimulation. Nervenarzt 73(4): 332-5.

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W (2003c) Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clinical Neurophysiol 114(4): 600-4.

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527(3): 633-9.

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57: 1899-901.

Nitsche MA, Paulus, W (2009) Noninvasive brain stimulation protocols in the treatment of epilepsy: current state and perspectives. Neurotherapeutics 6(2): 244-50.

Nitsche MA, Paulus W (2011) Transcranial direct current stimulation-update. Restor Neurol Neurosci 29(6): 463-92.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, Tergau F (2003d) Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. Journal of Cognitive Neuroscience, 15(4): 619-26.

Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, Fricke K, Liebetanz D, Lang N, Antal A, Paulus W, Tergau F. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 568: 291-303.

Parent A (2004) Giovanni Aldini: from animal electricity to human brain stimulation. Can J Neurol 31: 576-84.

Pascual-Leone A (2002) Handbook of Transcranial Magnetic Stimulation. London New York: Arnold; Oxford University Press.

Pascual-Leone A (2006) Disrupting the brain to guide plasticity and improve behavior. Prog Brain Res 157: 315-29.

Pascual-Leone A, Freitas C, Oberman L, Horvath JC, Halko M, Eldaief M, Bashir S, Vernet M, Shafi M, Westover B, Vahabzadeh-Hagh AM, Rotenberg A (2011) Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI. Brain Topography 24(4): 302-15.

Pascual-Leone A, Tarazona F, Keenan J, Tormos JM, Hamilton R, Catala MD (1999) Transcranial magnetic stimulation and neuroplasticiy. Neuropsychologia, 37: 207-17.

Pascual-Leone A, Tormos-Muñoz JM (2008) Transcranial magnetic stimulation: the foundation and potential of modulating specific neuronal networks. Rev Neurol 46(1): 3-10.

Patuzzo S, Fiaschi A, Manganotti P. (2003) Modulation of motor cortex excitability in the left hemisphere during action observation: a single-and paired-pulse transcranial magnetic stimulation study of self- and non- self-action observation. Neuropsychologia 41:1272-8.

Paulus W, Antal A, Nitsche M (2012) Physiological Basis and Methodological Aspects of Transcranial Electric Stimulation (tDCS, tACS, and tRNS), in: Miniussi, C., Paulus, W., and Rossini P. M. (Eds), Transcranial Brain Stimulation. CRC Press: 93-111.

Pavlova E, Kuo MF, Nitsche MA, Borg J (2014) Transcranial direct current stimulation of the premotor cortex: Effects on hand dexterity. Brain Res 1576:52-62.

Penfield W, Bolchey E (1937) Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60: 389–443.

Peña-Gómez C, Vidal-Piñeiro D, Clemente IC, Pascual-Leone Á, Bartrés-Faz D (2011) Down-regulation of negative emotional processing by transcranial direct current stimulation: effects of personality characteristics. PLoS One 6(7):e22812. doi: 10.1371.

Pereira JB, Junqué C, Bartrés-Faz D, Martí MJ, Sala-Llonch R, Compta Y, Falcón C, Vendrell P, Pascual-Leone A, Valls-Solé J, Tolosa E (2013) Modulation of verbal fluency networks by transcranial direct current stimulation (tDCS) in Parkinson's disease. Brain Stimul 6(1): 16-24.

Pisoni A, Gerfo EL, Ottone S, Ponzano F, Zarri L, Vergallito A, Lauro LJ (2014) Fair play doesn't matter: MEP modulation as a neurophysiological signature of status quo bias in economic interactions. Neuroimage, 101C:150-8.

Polanía R, Nitsche MA, Paulus W (2011) Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. Human Brain Mapping 32(8): 1236-49.

Priori A, Berardelli A, Rona S, Accornero N, Manfredi M (1998). Polarization of the human motor cortex through the scalp. Neuroreport. 9(10): 2257-60.

Priori A, Hallett M, Rothwell JC (2009) Repetitive transcranial magnetic stimulation or transcranial direct current stimulation? Brain Stimulation 2(4): 241-45.

Reis J, Robertson E, Krakauer JW, Rothwell J, Marshall L, Gerloff C, Wassermann E, Pascual-Leone A, Hummel F, Celnik PA, Classen J, Floel A, Ziemann U, Paulus W, Siebner, HR, Born J, Cohen LG (2008) Consensus paper: "Can tDCS and TMS enhance motor learning and memory formation?" Brain Stimul 1(4): 363–69.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer J, (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A 106(5): 1590-95.

Rioult-Pedotti MS, Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. Science 290: 533-36.

Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP (1998) Strengthening of horizontal cortical connections following skill learning. Nat Neurosci 1: 230-34.

Rothwell JC (1993). Evoked potentials, magnetic stimulation studies, and event-related potentials. Curr Opin Neurol 6(5): 715-23.

Rothwell JC (1997) Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. J Neurosci Methods 74(2): 113–22.

Rothwell JC (2011) Using transcranial magnetic stimulation methods to probe connectivity between motor areas of the brain Hum Movement. Science, 30: 906-15.

Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W (1999). Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol 52:97-103.

Sanes JN, Donoghue JP, Thangaraj V, Edelman RR, Warach S (1995) Shared neural substrates controlling hand movements in human motor cortex. Science 268(5218):1775-7.

Sanes J, Schieber M. (2001). Olderly somatotopy in primary motor cortex: does it exist? Neuroimage 13(6): 968-74.

Shum M, Shiller DM, Baum SR, Gracco VL (2011) Sensorimotor integration for speech motor learning involves the inferior parietal cortex Eur J Neurosci 34: 1817-22.

Sowman PF, Dueholm SS, Rasmussen JH, Mrachacz-Kersting N (2014) Induction of plasticity in the human motor cortex by pairing an auditory stimulus with TMS. Front Hum Neurosci 8:398. doi: 10.3389/fnhum.2014.00398.

Stagg CJ, Nitsche MA (2011) Physiological basis of transcranial direct current stimulation. Neuroscientist 17(1): 37-53.

Schwenkreis P, El Tom S, Ragert P, Pleger B, Tegenthoff M, Dinse HR (2007) Assessment of sensorimotor cortical representation asymmetries and motor skills in violin players. Eur J Neurosci 26(11): 3291-302.

Szczepanski SM, Saalmann YB (2013) Human fronto-parietal and parieto-hippocampal pathways represent behavioral priorities in multiple spatial reference frames. Bioarchitecture 3(5): 147–52.

Taubert M, Lohmann G, Margulies DS, Villringer A, and Ragert P (2011a) Long-term effects of motor training on resting-state networks and underlying brain structure. Neuroimage 57(4): 1492-98.

Taubert M, Villringer A, Ragert P (2011b) Learning-Related Gray and White Matter Changes in Humans: An Update. Neuroscientist 18(4): 320-5.

Tokuno H, Nambu A (2000) Organization of non primary motor cortical inputs on pyramidal and non pyramidal tract neurons of primary motor cortex: An electrophysiological study in the macaque monkey. Cereb Cortex 10: 58-68.

Turi Z, Ambrus GG, Ho KA, Sengupta T, Paulus W, Antal A. (2014) When size matters: large electrodes induce greater stimulation-related cutaneous discomfort than smaller electrodes at equivalent current density. Brain Stimul 7(3): 460-7.

Vahdat S, Darainy M, Milner TE, Ostry DJ (2011) Functionally specific changes in resting-state sensorimotor networks after motor learning. J Neurosci 31(47): 16907-15.

Veniero D, Ponzo V, Koch G (2013) Paired Associative Stimulation Enforces the Communication between Interconnected Areas. J Neurosc 33(34):13773-83.

Vicario CM, Komeilipoor N, Cesari, P, Rafal, RD, Nitsche MA (2014). Enhanced corticobulbar excitability in chronic smokers during visual exposure to cigarette smoking cues J Psychiatry Neurosci 39(4): 232-8.

Walther M1, Berweck S, Schessl J, Linder-Lucht M, Fietzek UM, Glocker FX, Heinen F, Mall V (2009) Maturation of inhibitory and excitatory motor cortex pathways in children. Brain Dev (7):562-7.

Ziemann U, Paulus W, Nitsche MA, Pascual-Leone A, Byblow WD, Berardelli A, Siebner HR, Classen J, Cohen LG, Rothwell JC (2008) Consensus: Motor cortex plasticity protocols. Brain Stimul 1(3):164-82.

Ziemann U, Rothwell JC, Ridding MC (1996) Interaction between intracortical inhibition and facilitation in human cortex. J Physiol 3: 873-8

8. ANNEXES

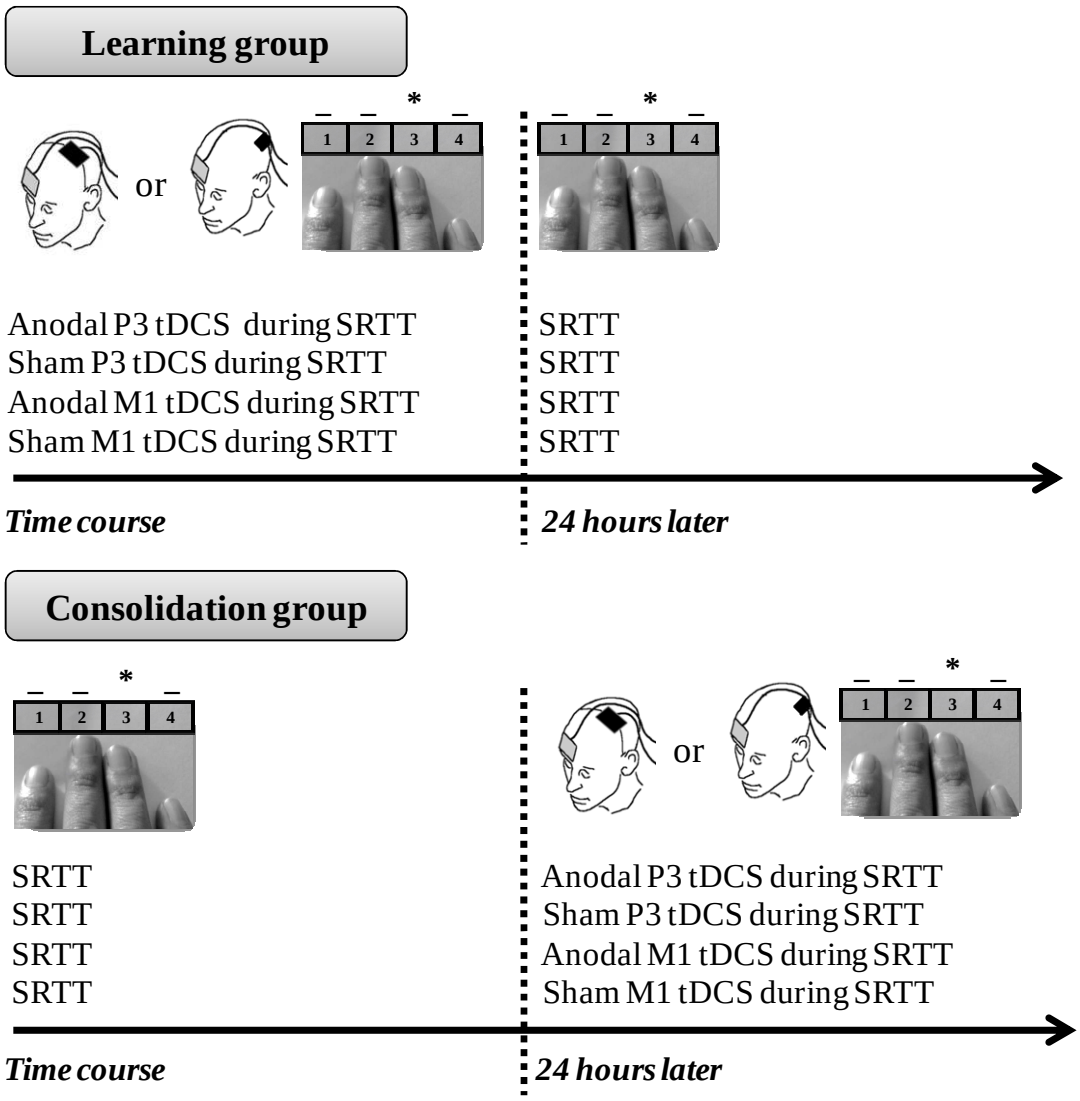
ANNEX I

Procedure for the Serial Reaction Time Task (SRTT) described by Kuo et al., 2008

"Subjects were seated in front of a computer screen at eye level and a response pad on the table with four buttons numbered 1–4. They were instructed to push each button with a different finger of the right hand (index finger for button 1, middle finger for button 2, ring finger for button 3, and little finger for button 4). An asterisk appeared in one of 4 positions that were horizontally spaced on a computer screen and permanently marked by dots. The subjects were instructed to press the key corresponding to the position of the asterisks as fast and correct as possible. After a button was pushed, the go signal disappeared. The next go signal was displayed 500 ms after the subject pushed the button, independent of correct or incorrect reaction. The learning test consisted of 8 blocks of 120 trials. In blocks 1 and 6 the sequence of asterisks followed a pseudo-random order in that asterisks were presented equally frequently in each position and never in the same position in two subsequent trials. In blocks 2–5 and 7 and 8, the same 12-trial sequence of asterisk positions was repeated 10 times (e.g. abadbcdacbdc). Subjects were not told about the repeating sequence, but asked after the last block of each session if they were aware of a repeating sequence, and if they were, to write it down. 4 versions of the serial reaction time task were generated, each subject received each version only once in randomized order to avoid interference effects".

ANNEX II

Time course of an experiment of our next project research



Subjects will participate in one of the following two groups: learning group and consolidation group. Four randomized different sessions will be held in each group (that will correspond to the two different stimulation conditions and the two different areas stimulated). For the learning group, posterior parietal cortex (P3) or primary motor cortex (M1) will be located according to the 10-20 electroencephalography international

system. Then, subjects will be stimulated with anodal or sham condition during the serial reaction time task performance through a computer. One day later, every subject will perform the task again without any stimulation condition. For the consolidation group, every subject will perform the serial reaction time task without stimulation. One day later, P3 or primary motor cortex will be located in each session according to the 10-20 electroencephalography international system, and then subjects will receive anodal or sham stimulation during the serial reaction time task performance. Four different versions of the task will be used for each group (one for each session). The specific version for each session into a group will be randomized between subjects.

ANNEX III

Curriculum vitae

PERSONAL DATA

Name: Guadalupe Nathzidy Rivera- Urbina
Place and date of birth: Mexico city, 26 – 06 - 1979

E mail : nathzidy@hotmail.com

ACADEMIC FORMATION

2008 - 2010 Master Neuroscience and Biology of the behavior

University Pablo de Olavide, Seville, Spain.

2006 - 2008 Master Neuropsychology

University Universidad Nacional Autónoma de México (UNAM)

1996 - 2000 Degree in Psychology. Homologated to the Spanish title of degree in Psychology.

University Universidad del Valle de México

PROFESSIONAL EXPERIENCE

2013 -2014 **Huelva University, Spain. (Universidad de Huelva).** Lecturer. Subjects: Psychological Basis for Special Education and Intervention in risk behavior.

2010 - 2011 **Huelva University, Spain. (Universidad de Huelva).** Lecturer. Subjects: Developmental Psychology and Psychological Basis for Special Education.

2006 -2008 **Medical Center of High Speciality UMAE HG National Medical Center "La Raza", Mexico.**

(Unidad Médica de Alta Especialidad UMAE HG Centro Médico Nacional "La Raza" IMSS). Neuropsychologist. Area: Pediatric Neurosurgery and Neurology.

REGIONAL HOSPITAL 1º DE OCTUBRE ISSSTE. Neuropsychologist. Area: Geriatrics.

2005 - 2006 **Mexica Clinic of Autism and Developmental Disorders. (Clñinica Mexicana de Autismo y Alteraciones de Desarrollo).** Therapist. Area: Infant (3-10 years old)

2004 –2007 **CÁRITAS**. Therapist. Area: Psychology.

2002 –2005 **Miguel de Cervantes Saavedra High schoolCOLEGIO MIGUEL DE CERVANTES SAAVEDRA IMECC** Teacher. Area: Psychology.

RESEARCH STAYS

Institution **University of Göttingen, Germany Neurophysiology Departament.**

July to September 2011 (3 months)

Institution **University of Göttingen, Germany Neurophysioly Departament.**

February to October 2013 (8 months)

POSTERS

Event **3rd International Workshop on Synaptic Plasticity: from bench to bedside. Milazzo Italy. 2014**

Title Parietal transcranial direct current stimulation affects primary motor cortex excitability in humans

Event **43rd European Brain and Behaviour Society Meeting. Seville Spain. 2011**

Title Hippocampal Lesions Disrupt The Tempo-Circadian Control on the Effect of Latent Inhibition of Taste Aversion Learning

Event **VII Congress of Neuropsychology in Andalucia, Spain. Andalucian Society of Neuropsychology. 2011**

Title Selective Neuropsychological effects associated with different lesions in the corpus callosum. A comparative study

Event **II Meeting of postgraduate students. UNAM, Mexico. 2008**

Title	Actualities in Neuropsychological Diagnosis
Event	**V Congress of Neuropsychology in Andalucia, Spain. Andalucian Society of Neuropsychology. 2008**
Title	Encephalitis: Neuropsychological Findings.
Event	**V Congress of Neuropsychology in Andalucia, Spain. Andalucian Society of Neuropsychology. 2008**
Title	Neuropsychological Assessment in a case of resection of right frontal hypothalamic pilocytic astrocytoma.
Event	**II Meeting of Neuropsychological Residence UNAM; México**
Title	Neuropsychological Assessment in a case of Alzheimer.

PAPERS

Title	**Parietal transcranial direct current stimulation modulates primary motor cortex excitability.**
Authors	**G. Nathzidy Rivera-Urina,** G. Batsikadze, A. Molero, W. Paulus, M. F. Kuo and M.A. Nitsche.
Journal	Submitted to *Brain Stimulation* (2014).
Title	**Down syndrome, brain and development ISSN: 0718-0446**
Authors	A. Molero and **G. Nathzidy Rivera-Urbina.**
Journal	Summa Psicologica 2013
Title	**Analysis of factors associated with cognitive decline in a sample in a population of geriatric. 43-60 ISSN: 0124-1265**
Authors	**G. Nathzidy Rivera-Urbina,** Gabriela Méndez Flores and Andrés Molero Chamizo
Journal	Revista Neuropsicología, Neuropsiquiatría y Neurociencias 2012
Title	**Brain and Behavior. A review ISSN:1852-4206**
Authors	Andres Molero Chamizo and **G. Nathzidy Rivera-Urbina.**
Journal	Revista Argentina de Ciencias del Comportamiento 2012

Title	**Neuropsychological and neurophysiological techniques: Contributions to the study of the biology of behavior ISSN:2172430X**
Authors	Andrés Molero Chamizo**, G. Nathzidy Rivera-Urbina** y Jason Lauder.
Journal	Avances de Neurología. 2010
Título	**Manuals and textbooks of psychopharmacology: a review ISSN: 0718-0446**
Authors	Andrés Molero Chamizo y **G. Nathzidy Rivera-Urbina.**
Journal	Summa Psicológica, 2010.
Título	**Encefalitis: hallazgos neuropsicológicos de un caso**. Abstract: V Congreso Andaluz de Neuropsicología.
Authors	**G. Rivera-Urbina**, J. Ruiz Chávez, J. Sosa Maldonado, S. Rojas y J. Bernal Hernández
Journal	Revista de Neurología, *in press*, Referencia: 2009230.
Título	**Evaluación neuropsicológica en un caso de resección de astrocitoma pilocítico hipotalámico frontal derecho**. Abstract: V Congreso Andaluz de Neuropsicología.
Authors	**G. Rivera-Urbina**, J. Ruiz Chávez, J. Sosa Maldonado, S. Rojas y J. Bernal Hernández
Journal	Revista de Neurología, *in press*, Referencia: 2009230.